DELVOCID® AND SODIUM CHLORIDE EFFECTS ON THE STABILITY OF A CORN BASED LIQUID SUPPLEMENT CONTAINING UREA, MONENSIN, VITAMIN A AND TRACE MINERALS

by

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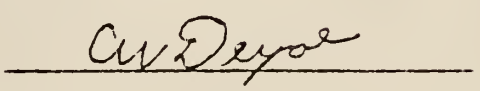

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INTRODUCTION

In recent years there has been a growing interest in the use of liquid supplements in cattle feeding due to their advantages over dry supplements. One advantage of liquids is the fact that they are easily absorbed by the dry ingredients in a complete ration due to their low viscosity and surface tension. Another major advantage of liquids is that they may be fed in diversified ways; (1) as a top dress on grain and silage; (2) from lick-tanks in a pasturing situation; (3) mixed as an ingredient in a complete ration. The storage and handling of liquid supplements may also be easier than dry.

Even though liquids have advantages over dry supplements there are still problems such as product uniformity, overconsumption, and product stability that present problems to the manufacture and user of liquid supplements. Many of the liquid supplements being fed today undergo a physical segregation of ingredients and nutrients. This may be visually observed but many times analytical techniques must be employed to determine the uniformity of these liquids. Overconsumption is always a major concern of livestock feeders when feed is fed on a free choice system. Chemical decomposition of nutrients presents a problem in liquids due to air oxidation and interaction of nutrients such as trace minerals and vitamins. This may be enhanced when these ingredients are fed in a liquid carrier.

In December 1975, Rumensin,[1] received Food and Drug approval to be used in the feeding of confinement cattle. The active ingredient of this additive is monensin sodium insin and will be referred to as monensin throughout the remainder of this

[1]Trademark, Elanco, Greenleaf, Indiana

paper. The benefit of the addition of this ingredient was an improvement of approximately 10% in feed efficiency. However clearance has not been given for the use of monensin in liquid supplements. If liquids are proven to be suitable carriers of monensin it is felt that cattle will respond in a similiar manner to this type of feed as they do to dry feed supplemented with Monensin.

The addition of Trace Minerals and Vitamins to liquid supplements would prove to be advantageous for many of the same reasons stated earlier for the use of liquids. By adding these micro-ingredients to liquids a more complete dispersion of these nutrients may be achieved through the improved mixing capabilities of liquid over dry supplements.

The purpose of this research was to investigate the use of a mold inhibitor (Delvocid®) in a corn based liquid supplement and then determine its effect on the products uniformity and stability. Investigation of the parameters necessary to stabilize monensin and soluble vitamin A and trace minerals in a corn based liquid supplement were also conducted. Urea was used as a non-protein nitrogen (NPN) source in the formulation of the corn based liquid supplement. Determination of crude protein, dry matter, starch damage, and several *in vitro* techniques were used to evaluate the effects of the mold inhibitor on the uniformity and stability of the liquid supplement. The stability of the vitamin A and trace minerals was investigated by determining the concentration of each micro-nutrient at different levels in storage tanks throughout a storage trial. Product uniformity was also investigated by determining crude protein, dry matter, and starch damage on samples taken

at different levels of the storage tanks throughout the trial.

LITERATURE REVIEW

History of Liquid Supplements and Their Ingredients

The term "liquid supplement" has been defined and used in the feed industry to describe a variety of feeds that are fed to livestock. These include any supplement that is made up of a liquid carrier to which a nitrogen source and other essential nutrients are added. The nitrogen source of these supplements may be one of the following types: protein nitrogen (ie: soybean meal or fish meal) or non-protein nitrogen (NPN ie: urea). The story of liquid supplements has paralelled the development and acceptance of urea as a NPN source for runinant animals.

Urea was first synthesized by Wohler in 1828 and the first reports of its use in ruminant feeding was by Zuntz in 1891. Throughout the next fifty years many papers were written on the use of urea in the ruminant and in monogastric nutrition as described by Stangel in 1963. These reports however demonstrated that urea was only utilized by ruminants through the synthesis of bacterial and protazoal protein by the rumen microflora. The full scale production of urea in the United States began in 1935 and the first studies of its use in this country was reported in the Digest of Research on Urea and Ruminant Nutrition (1958).

At about the same time that urea was being investigated as an ingredient in cattle, feed molasses was accepted as an ingredient in animal rations. The first report of the use of molasses in animal feed in this county was by Gulley et al (1890). As research on the utilization of urea continued it

was discovered that a readily available energy source was needed for maximum utilization of the urea NPN. Thus due to the high solubility of urea in water and water based liquids it was a natural conclusion to dissolve the urea into molasses and thus the idea of liquid supplements was born. The use of a molasses urea mixture had the following advantages: (1) the sugars in molasses provided a readily available energy source; (2) increased palatability of the feed due to the high palatability of molasses; (3) decreased dustiness of complete feeds when the molasses urea mixture was added to ground grain.

It was reported in Feedstuffs in 1958 that the use of alcohol increased the utilization of urea by the ruminant animals by functioning as hydrogen donors during the ruminant fermintation.

The estabilshment of phosphoric acid as a highly available source of phosphorous in animal feeds proved to be a step forward in the development of liquid supplements. Richardson and coworkers (1961) along with Tillman and Brethour (1958) have shown that phosphoric acid is comparable to dicalcium phosphate and steamed bone meal in supplying the phosphorous required by livestock. Due to some of the constituents of molasses a gel may be formed when mixing molasses with phosphoric acid. This normally occurs when the pH and temperature of the mixture is elevated. Weber and Miller (1965) demonstrated that the gel formation was eliminated upon the addition of small amounts of non-phosphatic acids such as sulfuric. They showed that the addition of sulfuric acid at a rate of 12 parts per 100 parts of 75% phosphoric acid on

a weight bases prevented gel formation. Phosphoric acid has been used in liquid supplements to repel insects, facilitate dissolution of urea, stabilize the product against fermentation, and to reduce the viscosity of the supplement. Anderson (1956) also claimed that addition of phosphoric acid increased vitamin stability due to the lowering of the pH.

In the late 60's and early 70's studies conducted at Kansas State University by Helmer and others (1970) showed that the processing of a starch-urea mixture through a cooker extruder increased the utilization of urea by ruminants. The gelatinization and structural damaging of the starch in this process allowed for better utilization of the carbohydrates during the fermintation and protein systhesis process of the rumen. Thus there was a lessening in the occurance of ammonia-toxicity by increasing the utilization of the NH_4^+ and allowing little NH_3 to escape the rumenal wall. Skoch (1976) and Binder (1976) investigated the possibility of a hydrothermally processed starch based liquid supplement containing urea. They determined that this product was stable and comparable with molasses based liquids and dry supplements.

Other studies by Huber (1972), Lofgreen and Otagaki (1960) have shown equal performance of dry and liquid supplements when fed to lactating cows. Studies by Arias et al (1951), Bell et al (1953) showed no consistant advantage of dry over liquid protein supplements.

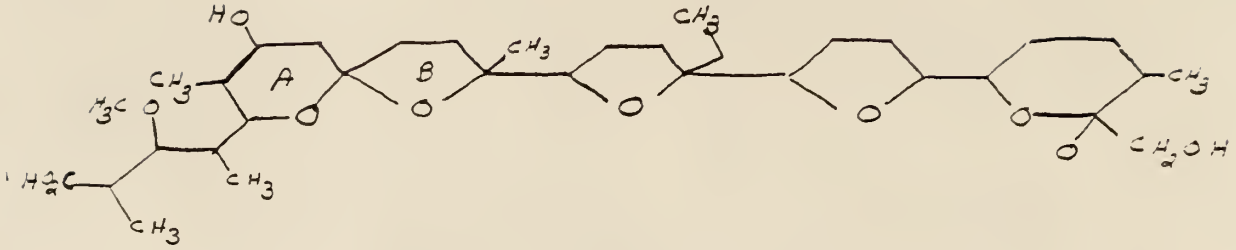
Na-Monensin

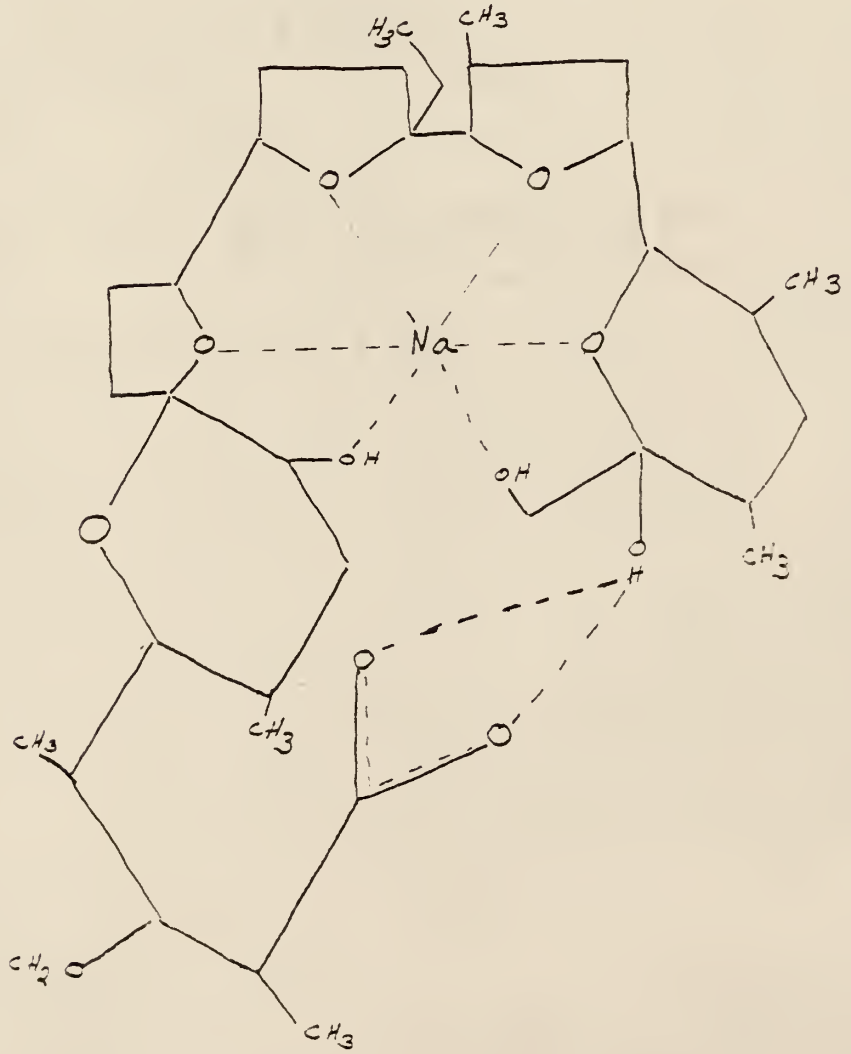
Monensin is a biologically active compound produced by a strain of Stretomyces Cinnamonensis which is effective in preventing coccidiosis in poultry with some activity against gram-positive organisms, Haney and Hoehn (1967). Figure 1 shows a planner representation of Monensin and of its sodium salt as described by Agtrarap and Chamberlin (1967). Monensin is a pentacylic compound with rings A and B forming a spiro-ketal moiety, rings C and D are substituted tetrehydrofuran rings and ring E is a cyclic hemiketal. Vicinal primary and tertiary hydroxal groups are found in ring E and a secondary hydroxal group is located in ring A. A single methoxyl group is found on the side chain bearing the carboxyl group. A total of eight methyl groups are present, one is a tertiary and another is part of an ethyl group.

In addition to its effectiveness against coccidiosis in poultry, Monensin has been shown to alter rumen fermentation by increasing the molar proportion of propionic acid. Work by Richardson and coworkers (1974) showed that a level of 1.0 ppm Monensin increased propionic acid production by 45% without affecting total volatile fatty acid production in vitro. Another study by these researchers has shown that feeding 200 mg/day in vitro increased proprionic acid molar percentage by 52%.

Potter et al (1974) has shown that steers and heifers grazing on grass-legume pasture had significantly lower ruminal molar percentages of acetic and butyric acid when Monensin was supplied. Campbell et al (1973) however reported that

FIGURE I: STRUCTURAL FORMULA OF
MONENSIN AND ITS SODIUM SALT





addition of Monensin to diets of grazing steers had little effect on the molar percentages of volatile fatty acids in the rumen. Utely et al (1975) and Hale et al (1975) showed that hay diets supplemented with small amounts of grain containing Monensin increased the molar percentage of propionic with a corresponding decrease in acetate and butyrate. Campbell et al (1973a), Wilson et al (1975), and Utely (1976) have all reported that Monensin supplementation of high-concentrate diets cause a small change in the volital fatty acid (VFA) production of the rumen. But there was a definite increase in propionate with a decrease in acetate and butyrate. Hale et al (1975) however observed that acetate values tended to increase while propionate values remained the same when Monensin was fed in combination with high-concentrated diets.

The mode of action through which Monensin alters the rumen fermentation and production of VFA is not completely understood. Work by Beede and Farlin (1975a, and 1975b) showed that Monensin did not reduce the in vitro lactic acid levels and therefore they postulated that the increased propionate production due to Monensin was through the acrylate metabolic pathway. Dinius and Simpson (1975) and (1976) found no effect on pH, dry matter digestion, crude protein digestion, or cellose digestion when varios levels of Monensin were fed. These workers showed a characteristic decrease in the acetate to propionate ratio but showed no effect on the number of protozoa, total bacteria, or cellulolytic bacteria in the rumen fluid obtained from animals fed up to 33 ppm of Monensin.

Monensin has been approved for use in feedlot rations for several years and recently it has been approved for use with range cattle. However it has not been approved for use in liquid supplements that are stored at pHs below 4.2. This is due to the chemical shift in the structure of Monensin under mild acid conditions to a spiroketal group of ring A, Agtarap and Chamberlin (1967).

However work by Cassler (1971) and by Choy et al (1974) have shown that Monensin and the other ionophors of this group of compounds are capable of transporting mono and divalent cations across membranes against concentration gradients. Work by Estrada-O et al (1972) showed that these compounds were also capable of moving Ca^{+2} and other ions across membranes against pH gradients.

Vitamin A:

McCollum and David (1915) were the first to postulate the existance of a "Fat Soluble A Factor". The structure of this factor was determined by Karrer and Coworkers (1931) and later synthesized by Kuhn and Morris (1937). Studies conducted by Steenback and Boulwell (1920) showed that naturally occuring carotenoids had vitamin A activity.

As discussed by Moore (1957) vitamin A and carotenoids are unlike most of the other known vitamins in that they can exist in a multitude of different forms. To complicate things even further each of these derivatives of vitamin A and carotenoids can exist in many isomeric forms and each of these isomers exhibits a different biological activity. These compounds are not soluble in water or glycols are slighty soluble

in methyl, ethyl, and propyl alcohols, and are extremely soluble in chloroform, cyclohexane, ether, fats, and oils, Windholz (1976). Vitamin A compounds and their solutions are inactivated quite rapidly by ultra-violet light and by oxygen. This decomposition is accelerated by rancid fats, trace minerals, moisture, and elevated temperature. In most cases oil solutions of Vitamin A are more stable than the pure crystals and the Vitamin A esters are more stable than vitamin A alsohols.

The difference in biological activity of vitamin A and carotenoids is believed to be due to the variation in the atomic arrangement of the side chains of these isomers. Table I lists the six isolated isomers of vitamin A and their activity and bio-potency. The all-trans isomer of vitamin A has been shown to exhibit the highest biological activity and is therefore used as the international standard and is given a value of 100% by definition. It can be seen from this table that all other vitamin A isomers have a lower bio-potency and activity. Also a mixture of any two of these isomers with the all-trans form will result in an activity lower than 100%. Table II gives the relative Bio-potencies of some major carotenoid isomers. B carotene is used as the international standard, all other isomers have a lower carotene conversion when determined by rat bioassay. These values will all be much lower for swine, poultry, and ruminants.

Biological availability is defined as the percent of the nutrient consumed by an animal that is actually absorbed and utilized by the body, compared to an appropriate standard.

TABLE I

Relative Bio-Potency of Vitamin A Acetate Isomers [1]

Isomer	Activity (USP Units/gr)	Bio- Potency
All-trans	2,904,000	100% *
13-cis (neo)	2,190,000	75%
9-cis	607,000	25%
9,13-di cis	688,000	24%
11-cis	690,000	24%
11,13-di cis	428,000	15%

TABLE II

Relative Bio-Potency of Carotenoid Isomers

Carotenoid	All-trans Isomer	Mono-cis Isomer
β -Carotene	100%	38%
α -Carotene	53%	13%
γ -Carotene	42%	19%
Cryptoxantjin	57%	27%

*By Definition

[1]Vitamin A, First Ed. Chas. Pfizer and Co., Inc.

The biological availability of vitamin A sources is controlled by two major factors; (1) the diluent, matrix or vehicle containing the vitamin A and; (2) the size of the vitamin A particle in the diluent or vehicle. It has been shown by Lewis et al (1950) and Sobel et al (1950) that vitamin A absorption is better from emulsified solutions of Vitamin A in water than from vitamin A oil solution. Thus the smaller the particle size of vitamin A and/or the easier vitamin A is removed from solution (water vs oil) the better animal absorption. These results were also shown in calves fed different particle sizes of vitamin A and different solvents, Jacobson et al (1954). The results showing the poor availability of vitamin A in oil and fat suspension has been shown to be due to the solvent and not due to the source of vitamin A itself.

The vitamin A requirements according to the National Research Council (1978), (1970) are 10-20, 000 units/head/day for fattening steers on corn and/or alfalfa, 20-30,000 units/head/day of vitamin A for fatting steers on grain sorghum and barley. This difference is due to the high vitamin A content of corn and alfalfa. Dairy cattle require 30,000 units/head/day while calves require 6-10 million units/head/day. These values are higher than the requirement for normal growth, this is due to one or a combination of the following; (1) Maximum growth rates rather than normal growth is required; (2) Greater stress conditions are generally present due to temperature variation, crowding, disease, and management lapses; (3) Averages are needed to compensate for potency losses due to manufacturing and storage techniques.

TRACE MINERALS

INTRODUCTION

There are many elements that occur constantly in living tissue in such minute amounts that the early workers were unable to quantitate their concentration and for this reason these elements became known as trace minerals. Studies of trace minerals began about a century ago when scientists became interested in compounds of limited biological significance, as reported by Underwood (1962) some of the early studies were conducted on compounds such as turacin, a porphyrin compound which contains copper found in the feathers of birds; hemocyanin which is another copper containing compound found in the blood of snails. These studies however gave little information on the role or function of these compounds in the body. Bernard (1857) through his studies of respiration and other oxidative processes was instrumental in the discovery of the metalloenzymes and the metal-enzyme catalysis.

In the 1920's investigation into the role of trace elements really got its start. Hart (1928) and coworkers showed conclusively the need for copper in the nutrition of rats. This gave a new stimulus to the study of trace elements and their deficiency as the cause of nutritional diseases in man and his domestic animals.

Trace elements have been classified into four divisions: (1) the essential; (2) the probably essential; (3) the toxic and (4) the physiological inactive elements. This paper will be limited to talking about the essential elements only. The essential trace minerals are those that meet the following

criteria: (A) reported demonstration of a significant growth response to dietary supplementation of the element and the element alone; (B) development of a deficiency on diets otherwise adequate and satisfactory; (C) correlation of the deficiency state with the occurance of subnormal levels of the element in the blood or tissue of animals exhibiting the response. The minerals that will be dealt with here are Iron, Iodine, Manganese, Copper, Cobalt, and Zinc which are the basic minerals that are required for ruminants.

IRON

The ancient Greeks regarded iron as having been imbued by Mars with the forces and strength and was capable of imparting these properties to people that suffered from weakness. Granick (1832) was the first to demonstrate iron deficiency in "chlorosis". Dawson (1902) demonstrated that inorganic iron cured anemia in "salt-sick" cattle. Dowale et al (1960) determined that the absorption of iron in rats occurs in three stages: (1) mucosal uptake; (2) mucosal transfer and; (3) mucosal storage. These same authors also showed that; (1) absorption of iron is more efficient when body stores are low; (2) the amount absorbed is only a small portion of that ingested; (3) absorption is directly into the blood; (4) most of the iron absorbed by the body takes place in the duodenal area of the small intestine. Even though most this work has been done in the rat it is believed by most workers that the same things happen in other species including ruminants.

Both ferric (Fe^{+2}) and ferrous (Fe^{+3}) forms of iron can be absorbed into the epithelial cell when the proper low-molecular weight chelating agents are present. Certain compounds such as ascorbic acid, HCL sugars, and polyals have been shown by Saltman (1965) and Pearson et al (1966) to increase the absorption of iron. Pearson et al (1966), Benson et al (1967) have shown that phytates, gastroferrin, endotoxins, alkalinizing agents and phosphates inhibit the absorption of iron. These workers have also shown the EDTA chelated iron is absorbed readily by the gastro-intestinal tract, however due to the strength of this bond this form of iron is secreated via the kidney without abosrption into the body. Other chelating compounds however, rapidly transfer their iron to apotransferrin as demonstrated by Benson and Rampone (1967) and by Rubin and Princiotto (1961). Examples of these chelating agents are ascorbic acid, amino acids, α-keto glutaric and kalp.

Work by Saltman (1965) has shown that iron does not exist as the free metal in the physiological process but is always hydrated or tightly bound to protein molecules. This phenomenon is true especially in the transport of iron in blood serum, with a compound known as transferrin being responsible for this transport.

Iron requirements for ruminants has been the subject of many research papers, however most of the work has been in the area of anemia. Blarter et al (1957), Matrone et al (1957), and Thomas et al (1954) have shown the iron requirements of dairy calves to be 30 to 50 mg/day when given intravenously and 60 to 75 mg/day if fed orally. From a practical standpoint, iron deficiency does not pose a problem in older ruminants that

are fed diets containing ingredients other than milk. Iron content of ingredients normally used in cattle rations as follows: (1) leguminous plants 200 to 300 ppm; (2) grasses 100 to 250 ppm on a dry basis; (3) oilseed meals 100 to 200 ppm; (4) cereal grains 30 to 60 ppm, as reported by Underwood (1962) Studies by Ammerman et al (1967) showed that Fe-dextron injected into the body proved to be most effecient form of iron. Other forms investigated in order of decreasing availability are ferrous sulfate, ferrous carbonate, ferric chloride, and ferric oxide.

IODINE

Iodine is a unique trace element since it is only found in a functional role in thyroid hormones, thyroxin and triiodothyroxin. This mineral also has a long and fascinating history. The early Greeks used burnt sponges as a treatment of goiter. In 1811 iodine was discovered in sponges and seaweed ash as reported by Underwood (1962). Physicians were using salts or iodine to treat goiter.

Work by Alexander et al (1968), Barva et al (1968) have shown that 10% of the inorganic iodine is absorbed in the gastrointestinal tract of man and cattle. The site of iodine absorption is not limited to one specific location of the G.I. tract but iodine is absorbed all along this tract. Once the inorganic iodine is absorbed it is conjugated by the liver and secreted into the bile, most of this organic iodine is reabsorbed. There is however some lost in the feces and urine. Once this organic iodine has been absorbed it enters the "iodine pool"

of the body which is composed of extracellular space, red blood cells, and the thyroid, salivary, and gastric glands, as reported by Berson (1970).

The iodine requirement for ruminant animals is dependent upon the type of production given by the animal. The requirements for lactating cows as reported by the NRC (1978) is .50 ppm of the complete ration, and the requirement for cattle is .25 ppm of the complete ration NRC (1978). The normal source of dietary iodine is drinking water which contains from .1 ppb to 2 ppb in goiterous areas and from 2 ppb to 15 ppb in nongoiterous areas. The use of plants for iodine supplementation is tremendously variable due to different plant species, soil types, and fertilization as reported by Hercus and Roberts (1915) and Orr et al (1914). Iodine may be supplemented by iodized sodium chloride or by potassium iodate.

MANGANESE

Underwood (1962) reports that manganese was first demonstrated to be present in plant and animal in 1913. This trace element is known to be required as a cofactor for many enzymes that function in oxidative phosphorylation, fatty acid synthesis, and cholesteral synthesis. Not much is known about the absorption of manganese from the gastrointestinal tract, however transportation of this mineral is in the trivalent state which is bound to one or more subunits of a protein called transmaganium.

The requirement of ruminants for manganese differs considerable for those that are growing and those that are re-

producing. Research by Rajas et al (1965) and by Bentley and Phillips (1951) have shown that the manganese requirement for lactating cow is 20 ppm. Whereas work by Anke (1966) and Embry et al (1958) has shown that young calves require 15-36 ppm of manganese for normal growth. Beef cattle require a manganese level of 10 ppm in their diet as reported by Bentley and Phillips (1951).

COPPER

It has been known for many years that copper is found in significantly high levels in plant and animal tissues. This occurance was believed to be accidental until it was shown that copper was an important cofactor in hemoglobin formation as reported by Hart et al (1928). Also copper has been shown to be a component part of enzymes related to oxidative functions of the body, examples are tyrosinase, ascorbic acid oxidase, cytochrome oxidase. Copper is also found complexed with protein in a compound known as hemocyprein in red blood cells in ceruloplasmin which is located in the blood plasma, Church and Pond (1974).

Dick (1954) has shown that copper absorption is related to the acidity of the surrounding and for this reason it is believed that it is absorbed in the upper portion of the intestine and in the stomach mucosa. Comar and his coworkers (1948), have shown that the liver contains 33% of the copper that has been administered by either oral or intravenous injections, whereas the blood contains 9.4% and the remain-

ing tissues of the body only 1%. The metabolism of copper is affected by the presence of other trace elements in the diet as described by Dick (1956). High levels of Molybdenum will cause a decrease in the uptake and metabolism of copper. An increase in the concentrations of sulfates and sulfites also will lead to poor metabolism.

Due to the detrimental effects of other trace minerals the dietary requirements of copper will change. Matrone et al (1957) and Dick (1956) have shown that diets containing low molybdenum and sulfate concentration the requirement is only 3.6 mg. of copper per day. The same researchers have also shown that in diets containing high levels of molybdenum and or sulfates the animals requirement is between 7.2 and 8.0 mg per day.

COLBALT

This trace element is also an unusual mineral as is Iodine since it also serves one function in the body. That is as a component part of vitamin B_{12}. Cobalt was first discovered in plant tissues as early as 1841 as reported by Church and Pond (1974). Underwood (1962) states that in 1935 work done in Austria proved conclusively that cobalt was an essential trace element.

A symbiotic relationship between the rumen flora and the host animal exist in the metabolism of cobalt. The microorganism use the cobalt to synthesize vitamin B_{12} which is required

by the animal for the transfer of methyl groups during catabolisum of certain amino acids. Besides vitamin B_{12} the microorganisms found in the rumen use cobalt to synthesize other compounds which apparently are not used by the animal. This fact may explain the high dietary requirement of this mineral as compared to other animals.

Church and Pond (1974) reported that young growing sheep have the highest daily requirement for cobalt that of 6.2 mg per day, followed by mature sheep and young calves at 0.08 mg per day. Mature cattle require 0.05 mg per day. It has been demonstrated by Davis and coworkers (1956) that diets that contain molybdenum at levels between 200-400 ppm require larger concentrations of cobalt due to the inhibiting effect of molybdenum on cobalt utilization. On the other hand work by Chapman (1957) has shown that cobalt increases the utilization of copper and iron in diets that contain either high or low concentrations of molybdenum.

ZINC

This trace element is believed to function in many ways in the body: (1) activator for enzymes; (2) association with insulin and; (3) required for the synthesis of ribonucleic acids. Only one of these functions has been proven and that is the role of zinc in the activation of enzymes.

Investigation by Miller and Cragle (1967) into the absorption and secretion of zinc in the gastrointestinal tract has shown that this trace element is absorbed in the abºma

sum and the lower portion of the small intestine. These same workers have shown that the ability to absorb zinc decreases with maturity. The absorption of zinc from the diet may be affected by: (1) concentration of calcium, Grashuis (1964) reported that for every .1% above .3% calcium increased in the diet zinc requirement of the diet increase by 16 ppm; (2) high levels of magnesium may cause reproductive disorders due to zinc deficiency; (3) high molybdenum concentration in the body causes a decrease in the zinc concentration of the bodies organs but an increase in the concentration in the blood and kidneys, Underwood (1962).

The dietary requirements of zinc are 10-14 ppm for young calves when no plant protein is supplied and 8.7 ppm when plant protein is supplied, Mills et al (1961). Work by the same researchers have shown that mature ruminants require 20-30 ppm on a dry matter basis. Studies on the concentration of zinc in different ingredients by Gladstone and Loneregan (1967) have shown that availability decreases with maturity in forage crops. The same studies showed that legumes and herbs contain higher concentrations of zinc than cereals and grasses. Miller et al (1967) have shown the availability of zinc in oxides and sulfate salt from to be high but more research is needed in this area.

EFFECT OF DELVOCID® ON THE STORAGE OF A CORN BASED LIQUID SUPPLEMENT

Introduction

Due to the high moisture content of liquid supplements mold growth presents a large problem in the storage of products. The major requirement for mold-inhibiting compounds is that they be non-toxic to the animal being fed. Wiggen (1958) and Rao (1972) have shown various inorganic and organic acids capable of preventing mold growth on high moisture grains. Skoch (1976) and Binder (1976) showed that .5% propionic acid was effective in preventing mold growth in starch based liquid supplements.

Delvocid® developed by Gist-Brocades of Delft; Holland is a colorless, crystal of high shelf life stability presently being used in Europe in the cheese industry. The major ingredient of Delvocid® is pimaricin a tetraene compound that is effective against nearly all molds and yeast, however it has no effect on viruses, bacteria, and other microorganisms as reported by Mol (1966). For this reason this product should be acceptable for use in liquid supplements fed to ruminants, because it is capable of inhibiting mold growth but will not interfere with the microflora found in the rumen.

This research was designed to determine the level of Delvocid® needed to prevent chemical changes during an 8 week

*®Gist-Brocades Industrial Products Division p.o. box 1, Delft, Holland.

storage trial of a corn based liquid supplement, containing urea.

MATERIALS AND METHODS

Hydrothermal Processing:

The experimental liquid supplements were processed using a modified Penick and Ford laboratory[1] continuous jet cooker. The cooker is equipped with a Moyno pump calabrated to deliver 1.7 liters of slurry to the hydrothermal heater per minute. Live steam is injected into the slurry through the hydrothermal heater allowing a maximum temperature of 163° and a pressure of 65 psig to be achieved. The cooker is equipped with temperature and pressure gauges to allow regulation of these processing variables. This type of processing causes extensive shearing and cooking of the starch.

Processing of Liquid Supplement Samples:

Table 1 gives the formula of a 1:1 (corn to urea on an dry basis) liquid supplement formulated to contain 60% protein equivelent. The corn, urea, and water (added before cooking) were blended together, to give 20 kg of final product. This slurry was then processed through the hydrothermal cooker described above, at 150° and 55 psig at a rate of 2 27 kg per minute. Water was absorbed due to the condenation of steam during the cooking process After cooking {-amylase was added at a rate of .12% of the starch content at a temperature of 70° and allowed to react for 14 min.

[1]Penick and Ford, Ltd. Cedar Rapids, Iowa.

At the end of this reaction time the enzyme activity was terminated by the addition of 1% phosphoric acid. The product was then cooled to 45°C and the pH adjusted to 3.7 with additional phosphoric Acid (75% feed grade). The product was then divided into 3kg samples and placed in 3.8 liter plastic containers. Delvocid and propionic acid treatments were added at the levels shown in Table 2. Samples JD 5 A&B and JD 6 A&B were subdivided into four samples of 250 g each and placed in wide mouth mason jars and pressure cooked at 5 psig for 5 min. These samples were used as positive and negative controls respectively throughout the storage trials. The plastic containers and mason jars were then stored at room temperature.

Samples of each treatment were taken at 0,2,4, &8 weeks during the trial and analysis run to detect variation within and among treatments. Factors used to evaluate the effect of Delvocid levels on the storage of this supplement were: (1) pH; (2) crude protein; (3) dry matter; (4) starch damage C mg maltose/g of supplement; (5) free ammonia (NH_3-N); (6) viscosity and: (7) protein synthesis.

pH

A Beckman pH meter was used to determine the pH of the liquid supplement to the nearest hundreth of a pH unit. Results are given in Table I of Appendix A.

Crude Protein

The A.O.A.C. (1975) boric acid modification of Macro-Kjeldahl was used to determine the nitrogen content of the samples. Duplicate samples were run with the nitrogen content

of the samples. Duplicate samples were run with the nitrogen content being multiplied by 6.25 to give the protein equivelant. Weekly determination are reported in Table II of Appendix A.

Dry Matter

The official A.O.A.C. (1975) method for moisture was used. This involves the drying of a 2g sample for 1 hr at 130° C and from this the percent dry matter was calculated. Samples were run in duplicate and the weekly results are reported in Table III of Appendix A.

Starch Damage

Sung's (1968) method for starch damage which is based on Sandsted and Mattern (1960) method of hydrolysis with B-amylase with subsequent determination of reducing sugars by ferriccyinide was modified for this analysis. The modification was in reducing the B-amylase to 60 mg per 1g of sample digested, this allowed for an indication of the amount of readily degradable carbohydrates present in the liquid supplement. Results are reported as mg of maltose per gram of dry matter in Table IV of Appendix A. Variation due to daily determination was eliminated by comparison of determination to a maltose standard.

Free Ammonia

A micro-diffusion technique as described by Conway (1962) was used to determine the amount of free ammonia present in the liquid supplement samples. This method involves the

liberation of ammonia by potassium carbonate which is then trapped by a standard boric acid solution. The ammonia is then titrated with standard HCl. Table V, of Appendix A, contains the weekly determination reported as mg of free amonia (NH_3-N) per gram of dry matter.

Viscosity

Viscosity of the samples was determined at room temperature (25 to 27°C) using a LVT Brookfield Viscometer[1]. Comparison can only be made on viscosities taken with the same spindel and rpm. For this research spindel number 2 and a rpm setting of 12 was used and the results of these determinations are given in Table VI of Appendix A in centipose (cps).

Protein Sythesis

Barr's (1974) in-vitro rumen fermentation technique was used to determine the amount of protein synthesized from the liquid supplement. Rumen fluid samples were collected from fistulated Angus steer that was adapted to a urea containing diet. Fluid was collected from all portions of the rumen by way of a fistula, strained through two layers of cheese cloth into a thermos and mixed. This sample was then taken to the lab where it was strained immediately through four layers of cheese cloth. Ten milliliters of this strained fluid was then introduced by way of a 20 ml syringe into a 50 ml centrifuge tube that contained .26g of liquid supplement and .9g of corn though a 4/64 in hammer mill screen. The use of this mixture

[1]Brookfield Engineering Laboratories, Inc., Stoughton, Mass.

of liquid supplement and ground corn was to simulate a 16% crude protein complete feed.

To the rumen fluid and sample was added 20 ml of a 6.8 pH buffer that contained electrolytes similiar to saliva. The oxygen in the tubes was then evacuated with carbon dioxide and immediately closed with rubber stoppers equipped with a busen valve. Feed blanks were also prepared by taking 0.26 g of liquid and 0.9 g 4/64 ground corn and adding 30 ml of buffer. These tubes were also evacuated with CO_2 and stoppered. The feed samples and feed blank tubes were then incubated in a 39°C water bath for four hours. At the end of this incubation period the tubes were centrifuged at 25,400 x G for 15 minutes. The supernatant was discarded and the residue was rinsed twice with 25 ml of methanal used for each wash. The remaining precipitate was then transferred to a Kjeldahl flask and the nitrogen content was determined. Rumen fluid blanks were prepared by taking 10 ml of rumen fluid and adding 20 ml of the buffer in a centrifuge tube. These tubes were not incubated prior to centrifugation and washing.

The milligrams of protein per feed sample, feed blank, and fluid blank was calculated as follows: mg protein = ml acid x 14 x 6.25. Milligrams of protein synthesized by the liquid supplement samples were calculated by the following formula;

mg protein = mg protein in feed sample - (mg protein in feed blank + mg protein in fluid blank).

Table VII and VIII of Appendix A gives the weekly determination of protein synthesis reported as a percent of the negative and positive controls on a dry matter protein bases.

Statistical Analysis:

SAS 76 developed by Barr et al (1976) is a canned statistical analysis program that was used to analyze the results of the analysis mentioned in a split-plot design. Differences between Treatment Means, Week Means, and Treatment by Week interaction Means was determined. The analysis of Variance (AOV) tables for each dependent variable is found in Appendix D.

Results and Discussion

This study was designed to determine the optimum level of Delvocid^{R} that would prevent chemical change in a starch based liquid supplement during storage. Controls for this experiment were .5% propionic acid (samples 5 A&B) as a positive control and no preservatives added (samples 6 A&B) as a negative control. The .5% propionic level was used due to the results of Binder (1976) and Skoch (1976) who used this preservative with starch based supplements. Samples 4 A&B were used in this study to compare the Delvocid® treated samples to a propionic sample that had been stored under the same conditions.

The statistical analysis was conducted on a split-plot design with the treatments being the whole plot and weeks as subplots.

Effect of Storage of pH:

Table 1 of Appendix D gives the analysis of variance (AOV) table for the dependent variable pH during the storage study. It can be seen that treatments, weeks of storage, and the interaction between these two independent variables were significant. An examination of the data showed that most of the interaction was due to treatment 6 (the negative control). Removing this treatment from the analysis gave the AOV table given in Table 2 in Appendix D. This showed that at the $p<.05$ level the significant interaction was due to treatment 6, this allows one to compare the remaining treatments over time. Table 3 gives the average of each treatment per week, the trial treatment means, the weekly means of all treatments, and the weekly means with treatment 6 removed.

There was a significant difference between week 0 and 2 for all treatments with no significant change during the remainder of the trial for all treatments. Treatments 5 (positive propionic acid, pressure cooked control) and treatment 3 (250 ppm Delvocid®). The other treatments had a larger increase in pH during the first two weeks of storage and then leveled off with no further significant change. Comparison of the averages of each treatment over the entire trial shows that 5 again had the lowest pH value and that treatments 5 and 3 were close behind even though treatment 3 was found to be different ($p<.05$) from 5 mathmatically it can be seen that physiologically there is no difference between these treatments. Treatment 1 and 2 on the other hand showed an increase of greater

than .1 pH units which could be physiologically and significantly different since the reason for reducing the pH down to 3.7 was to control consumption of the corn based liquid supplement.

Crude Protein:

The results of the statistical analysis of the dependent variable crude protein (as is) is given in Table 3 of Appendix D. The average of crude protein data is given in Table 4 along with the treatment means and weekly means. It can be seen from the AOV table that there was no difference ($p<.05$) between the treatments but there was a difference between weeks of the trial. This difference is found in week eight of the trial where there was a 1% increase in crude protein content of the liquid supplement. To determine if this difference was due to a chemical (microbial growth) change in the product or an artifact of a change in dry matter composition, the crude protein values were corrected to a dry matter basis. The results of the statistical analysis are given in Table 4 of Appendix D. Table 5 gives the treatment means by week - the weekly means and the treatment trial means. When the protein is expressed on a dry matter basis it is seen that the weeks are again different ($p<.05$). An examination of the means of each treatment by week combination weekly averages, and treatments when averaged over the entire trial. An examination of the means of individual weeks however does show differences ($p<.05$). This is due to the weekly variation in the determination

of crude protein. The results of this study can then be interpreted to indicate that there was no loss of nitrogen during this study.

DRY MATTER

The average of duplicate dry matter determination is given in Table 6 with the statistical analysis of this dependent variable given in Table 5 of Appendix D. This data shows the effect of Delvocid® on dry matter content of a corn based liquid supplement. The AOV table shows there was no difference ($p<.05$) between treatment means, weekly means, or treatments. Table 6 of these means, it is seen that none of the treatments had a significant effect ($p<.05$) on the dry matter content of the supplement when stored in containers that eliminate rapid evaporation of water. Thus the dry matter of this supplement will remain constant for eight weeks when stored in a licktank designed of the tank as such that rapid evaporation is controlled. This, along with the protein data indicates that there is little or no microbial activity in the supplement since no change occured in the content of these dependent variables. If there was microbial activity one would expect a decrease in dry matter and in protein due to the use of the dry matter as energy and the protein as nitrogen (for protein synthesis). Dry matter would have been lost through gaseous end products (CO_2, CH_4 ect.) of metabolism and crude protein could have decreased due to the use of the urea (NH_4^+) as a nitrogen source to build larger molecules causing a dilution of the crude protein.

Starch Damage:

Starch damage was used to determine the amount of readily available carbohydrates in the corn based liquid supplement, and is expressed in mg of maltose lg of supplement or per g of dry matter. This analysis is performed by using B-amylase digestion with a ferricyanide titration of reducing power. Thus the results of this analytical technique gives not only the amount of starch susceptible to B-amylase but also includes any reducing sugars also present in the liquid supplement. The results of the effect of Delvocid^{R} on this dependent variable are given in Tables 7 and 8 with the corresponding AOV tables being in Appendix D (Table 6&7). Table 7 gives the results of maltose reported on an as is basis (mg maltose lg of supplement) where as Table 8 gives the results when the maltose is corrected to a dry matter basis (mg maltose lg of dry matter). In both cases there was no difference ($p<.05$) between the treatments or the weeks of storage. This indicates that again there was no no effect on this dependent variable due to the Delvocid^{R} treatments. This is an indication of no microbial activity since most organisms that would infect this supplement would either contain B-amylase enzymes or would use the reducing sugars as energy sources and thus cause a decrease in the maltose containt over time.

Free Ammonia

Tables 9 and 10 of Appendix D gives statistical analysis of this dependent variable and Table 9 and 10 gives the agerages of replication by treatment and weekly means of free ammonia expressed on a "as is" basis (Table 9) and on a dry matter

basis (Table 10). This analytical technique gives the amount of ammonia nitrogen (NH_3-N) released per gram of liquid supplement when mixed with saturated potassium carbonate (Conway 1962). Results are reported as averages of duplicate determinations of samples taken from replications A and B as mg of NH_3N/g of supplement or as mg NH_3-N/g of dry matter. Upon examination of these results on a weekly basis it is seen that treatment 1 (150 ppm of Delvocid®), treatment 2 (200 ppm of Delvocid), and treatment 5 (5000 ppm propionic acid) had no change ($p<.05$) in the amount of free ammonia present in the supplement. Treatment 3 (250 ppm of Delvocid®) on the other hand had a slight increase in free ammonia during week 2 but then had decreased ($p<.05$) from the 2nd to the 8th week of the trial with no difference ($p<.05$) between the 4th and the 8th week samples. Treatment 4 (500 ppm propionic acid not pressure cooked) showed a increase ($p<.05$) in free ammonia in the first two weeks of storage with a decrease ($p<.05$) during the 4th week to the original amount found at the beginning of the trial. Treatment 6 (negative control) however showed a increase ($p<.05$) for all samples taken during the trial.

These results lead one to the conclusion that propionic acid and Delvocid® tend to devrease the amount of free ammonia nitrogen during storage. The mode of action of these preservatives are not known but it is believed that the NH_4^+ ion may be tied up as a component part of a salt of propionic acid and that it may be complexed with the Delvocid®. This complexation holds the ion in solution and thus keeps it from

being released to the atmosphere. These results also indicate that all three levels of Delvocid® are capable of stabilizing the release of NH_3-N during storage of this liquid supplement.

Further information into the amount of free ammonia present in this liquid supplement was determined by expressing the free ammonia on a dry matter basis. Table 9, Appendix D, gives the statistical analysis for free ammonia on a dry matter basis expressed as mg NH_3-N/g of dry matter. The analysis of variance shows that there was a difference ($p<.05$) between treatments during the storage study however there was also a difference ($p<.05$) in weekly determination and in the treatment by week interaction. To remove the significant interaction another analysis of variance was run with treatment 6 removed. This analysis showed that the interaction was due to this treatment alone. Thus differences between treatments and the weekly difference could be compared.

Investigation of treatment means on a weekly basis shows no difference ($p<.05$) in weekly determination for treatments 1, 2, and 5. While there was a decrease ($p<.05$) in free ammonia in treatment 3 during the 4th and the 8th week of the trial Treatment 4 again showed a increase ($p<.05$) in free ammonia during the 2nd week but then decreased back to the original level during weeks 4 and 8. Treatment 5 showed a increase ($p<.05$) during all weeks of the trial. Again it may be concluded that all levels of Delvocid® retarded the release of NH_3-N during storage of the liquid supplement.

Viscosity:

Viscosity is a measurement of the flowability of a liquid. Table 10 of Appendix D gives the analysis of variance for this dependent variable and Table 11 of this section gives the treatment and weekly means during the storage trials. The analysis of variance shows that there were no difference ($p<.05$) between the treatments or in the treatment by week interactions. However there was a significant difference in the weekly averages of this trial all treatments increased in viscosity from week 0 to week 2 of the trial and decreased some what until week 4 and then increased again. This pattern may be due to equipment error in the determination of this dependent variable however it may be concluded that the preservative had no negative effect on the gelling capacity of this liquid. Moreover this liquid did remain stable to gelling and separation during this 8 week storage study.

Protein Synthesis:

This in vitro technique is designed to determine the ability of rumen microorganism to change a plant or non-protein nitrogen (NPN) source and a carbohydrate source into microbial protein. As described earlier this procedure was run at weeks 0,2,4, & 8 of the trial with duplicate runs made using rumen fluid from each of two animals. In analyzing this variable two things had to be considered: (1) the variation between animals and (2) week to week variation in the rumen

fluid. These questions were adjusted for by running analysis on a weekly basis and determining if the estimation of variance of each weekly analysis was estimating the same error. If indeed this was the case one may pool the individual determinations and determine significant differences over the whole trial. The hypothesis that all estimates 6^2 are equal is determined by the Hartleys's F Max Test (Snedecor & Cochran, 1974). This test showed that the individual weekly estimates of variance were estimating the same error thus the data could be pooled. Table 11 of Appendix D gives the analysis of variance for this dependent variable. The protein synthesis is expressed on a dry matter protein basis to remove variation in dry matter and protein from this dependent variable. To put the dry matter protein synthesis on a common value treatment 5 (pressure cooked, 5000 ppm propionic acid) and 6 (pressure cooked, no preservative added) were each used as a control. This was done in order to express each treatment as a percent of these controls for comparison between treatments.

Table 12 gives the weekly protein synthesized as a percentage of treatment 5 and Table 13 gives the weekly protein synthesized as a percent of treatment 6. There were no differences ($p<.05$) between any of the treatments throughout the trial. Therefore it can be said that there was no detremental effect of Delvocid^R at any level of study on protein synthesis.

Summary

Delvocid®, a product of Gist-Brocades, presently being used in Europe as a mold inhibitor in cheese processing, was investigated as a possible preservative for a corn based liquid supplement. The supplement was formulated to contain 60% protein equivalent with a 1:1 ratio of corn to urea on a dry matter basis. Delvocid® was used at 0,150,200, & 250 ppm and compared to propionic acid at 5000 ppm. These supplements were compared during on eight week storage study. Variables looked at were pH, crude protein, dry matter, starch damage, free ammonia, viscosity, and protein synthesis. There was a significant increase in pH of all treatments during the first 2 weeks of storage. This increase amounted to approximately 0.1 pH units in all treatments except the negative control. No further change in this variable except in the negative control (pressure cooked no preservative added). Thus Delvocid^{R} was capable of maintaining the acid pH of the supplement as well as propionic acid.

There was no significant difference ($p<.05$) in dry matter, crude protein, or in starch damage during storage which indicated no microbial growth since microbes would have decreased the amount of dry matter and maltose present by utilization of this constituents for growth. There was however a difference ($p<.05$) during storage in free ammonia however except for treatment 6 (no preservative). There was only a .223 mg NH_3-N/g of sample difference in any of the treatments thus there were no practical differences in these values. There was a differ-

ence ($p<.05$) in the viscosity of these supplements during the first 2 weeks of the study, however there was no difference ($p<.05$) between the treatments.

In Vitro protein synthesis results indicate no difference ($p<.05$) between any of the Delvocid® treatments and the propionic acid control or the control with no preservative added. Therefore these results indicate that Delvocid® had no detrimental effect on nitrogen utilization from these supplements.

TABLE 1. CORN BASED LIQUID SUPPLEMENT FORMULAS

Ingredients	Percent As Is
Corn	25.85
Urea	23.71
H_2O (added before processing)	41.16
H_2O (absorbed during processing)	8.28
Phosphoric Acid (75% Food Grade)	1.75
Enzyme[1] (α- amylase)	.12

[1]Novo Laboratory Inc., Wilton, Conn.

TABLE 2. LEVEL OF MOLD INHIBITOR USED IN STORAGE EVALUATION

Treatments	Proprionic Acid (ppm)	Delvocid® (ppm)
1 A&B	-	150
2 A&B	-	200
3 A&B	-	250
4 A&B	5000	-
5 A&B[1]	5000	-
6 A&B[1]	-	-

[1]These samples were pressure cooked at 5 psig of pressure for 5 min. and served as the controls throughout the trial.

TABLE 3. EFFECT OF DELVOCID® ON THE pH[A] OF A CORN BASED LIQUID SUPPLEMENT DURING STORAGE

Treatment[B]	Week of Storage 0	2	4	8	Treatment Average
1 A&B	3.72[a]	3.83[b]	3.82[b]	3.82[b]	3.80[h]
2 A&B	3.73[a]	3.84[b]	3.84[b]	3.83[b]	3.81[i]
3 A&B	3.74[a]	3.81[b]	3.82[b]	3.82[b]	3.80[h,j]
4 A&B	3.72[a]	3.82[b]	3.81[b]	3.81[b]	3.80[j,k]
5 A&B	3.70[a]	3.81[b]	3.81[b]	3.81[b]	3.78[k]
6 A&B	3.71[a]	4.82[b]	5.31[c]	5.40[d]	4.81[l]
Weekly Means	3.72[a]	3.98[a]	4.07[b]	4.08[c]	
Weekly Means With Treatment 6	3.72[a]	3.82[b]	3.87[b]	3.19[b]	

[A] pH values are reported as averages of duplicate determinations.

[B] Treatment 1 (150 ppm. Delvocid®), Treatment 2 (200 ppm. Delvocid®), Treatment 3 (250 ppm. Delvocid®), Treatment 4 (5000 ppm. Propionic Acid), Treatment 5 (5000 ppm. Propionic Acid pressure cooked), Treatment 6 (no inhibitor cooked).

[a,b] Row mean averages with same superscript (a,b) are not different ($p<.05$).

[h,i] Column means with the same superscript (h,i) are not different ($p<.05$).

TABLE 4. THE EFFECT OF DELVOCIDED® ON THE CRUDE PROTEIN CONTENT[A], AS IS BASES, OF A CORN BASED LIQUID SUPPLEMENT

Treatment[B]	Week of Storage 0	2	4	8	Treatment Average
1 A&B	65.50[a]	65.47[a]	65.73[a]	65.85 [a]	65.88
2 A&B	65.68[a]	65.38[a]	65.30[a]	66.38 [a]	65.68
3 A&B	65.87[a]	64.71[a]	65.49[a]	66.71 [a]	65.69
4 A&B	65.63[a]	64.65[a]	64.78[a]	66.28 [a]	65.33
5 A&B	64.65[a]	65.88[a]	65.20[a]	66.33 [a]	65.51
6 A&B	64.71[a]	65.09[a]	65.22[a]	65.14 [a]	65.03
Weekly Means	65.337[a]	65.194[a]	65.384[a]	66.28 [b]	

[A]Crude Protein reported on an as is bases.

[B]Treatment 1 (150 ppm. Delvocid®), Treatment 2 (200 ppm. Delvocid®), Treatment 3 (250 ppm. Delvocid®), Treatment 4 (5000 ppm. Propionic Acid), Treatment 5 (5000 ppm. Propionic Acid pressure cooked), Treatment 6 (no inhibitor cooked).

[C]No difference ($p<.05$) between treatment means

[a,b]Row means with the same superscript (a,b) are not significantly different ($p<.05$).

TABLE 5. THE EFFECT OF DELVOCID® ON THE CRUDE PROTEIN[A], ON A DRY MATTER BASES, OF A CORN BASED LIQUID SUPPLEMENT DURING STORAGE

Treatment[B]	Week of Storage 0	2	4	8	Treatment Average
1 A&B	155.22[a]	153.975[a]	157.705[a]	158.965[a]	156.466[h]
2 A&B	156.87[a]	156.050[a]	157.750[a]	160.995[a]	157.916[h]
3 A&B	157.170[a]	155.980[a]	157.575[a]	158.32[a]	157.261[h]
4 A&B	157.120[a]	155.345[a]	155.305[a]	159.230[a]	156.750[h]
5 A&B	154.280[a]	154.555[a]	155.945[a]	158.685[a]	155.866[h]
6 A&B	154.095[a]	154.590[a]	154.500[a]	158.055[a]	154.810[h]
Weekly Means	155.792[a,b]	155.082[a,b]	156.463[a]	158.708[c]	

[A]Crude protein reported as the average of duplicates on a dry matter bases

[B]Treatment 1 (150 ppm. Delvocid®), Treatment 2 (200 ppm. Delvocid®), Treatment 3 (250 ppm. Delvocid®), Treatment 4 (5000 ppm. Propionic Acid), Treatment 5 (5000 ppm. Propionic Acid pressure cooked), Treatment 5 (no inhibitor cooked).

[a,b]Row means with the same superscript (a,b) are not different ($p<.05$).

[h,i]Column means with the same superscripts (h,i) are not different ($p<.05$).

TABLE 6. THE EFFECT OF DELVOCID ON THE DRY MATTER[A] OF A CORN BASED LIQUID SUPPLEMENT DURING STORAGE

Treatment[B]	Week of Storage 0	2	4	8	Treatment Average
1 A&B	42.2[a]	42.5	42.7	42.1	42.1[b]
2 A&B	41.9	41.9	41.4	41.2	41.6
3 A&B	41.9	41.5	41.6	42.1	41.7
4 A&B	41.8	41.6	41.7	41.6	41.8
5 A&B	41.9	42.2	41.8	41.8	41.9
6 A&B	42.0	42.1	42.2	41.7	42.0
Weekly Means	41.9[a]	42.0	41.7	41.8	

[A] Dry Matter is reported as the average of duplicate determination of each replication.

[B] Treatment 1 (150 ppm. Delvocid®), Treatment 2 (200 ppm. Delvocid®), Treatment 3 (250 ppm. Delvocid®), Treatment 4 (5000 ppm. Propionic Acid), Treatment 5 (5000 ppm. Propionic Acid pressure cooked) Treatment 6 (No inhibitor cooked).

[a] No difference ($p<.05$) between row means.

[b] No difference ($p<.05$) between column means.

TABLE 7. THE EFFECT OF DELVOCID® ON STARCH DAMAGE[A], ON AN AS IS BASIS, OF A CORN BASED LIQUID SUPPLEMENT DURING STORAGE

Treatments[B]	Week of Storage 0	2	4	8	Treatment Average
1 A&B	96.7[a]	104.8	95.0	97.3	98.4[b]
2 A&B	97.9	97.5	95.7	95.5	96.6
3 A&B	98.5	98.5	98.8	96.6	98.1
4 A&B	96.4	96.3	96.0	94.5	95.8
5 A&B	97.1	99.3	95.2	97.4	97.3
6 A&B	99.1	99.1	98.5	98.9	98.9
Weekly Means	97.6[a]	99.2	96.7	96.5	

[A] Starch Damage (mg maltose/g of supplement) are reported as the average of duplicate runs of each replication.

[B] Treatment 1 (150 ppm. Delvocid®), Treatment 2 (200 ppm. Delvocid®), Treatment 3 (250 ppm. Delvocid®), Treatment 4 (5000 ppm Propionic Acid), Treatment 5 (5000 ppm. Propionic Acid pressure cooked) Treatment 6 (No inhibitor cooked).

[a] No difference ($p<.05$) between row means.

[b] No difference ($p<.05$) between column means.

TABLE 8. THE EFFECT OF DELVOCID® ON STARCH DAMAGE[A], ON A DRY MATTER BASES, DURING STORAGE

Treatments[B]	Week of Storage 0	2	4	8	Treatment Average
1 A&B	229.2[a]	246.3	227.9	231.3	233.7[b]
2 A&B	233.8	232.7	231.2	231.5	231.3
3 A&B	234.9	237.5	237.9	229.3	234.8
4 A&B	230.8	231.3	230.2	227.0	229.8
5 A&B	231.7	235.1	236.1	233.0	234.8
6 A&B	240.9	235.4	234.4	236.9	236.9
Weekly Means	233.5[a]	236.4	232.9	231.5	

[A]Starch Damage Values (mg of maltose/g of dry matter) are averages of duplicate determinations of two replications.

[B]Treatment 1 (150 ppm. Delvocid®), Treatment 2 (200 ppm. Delvocid®), Treatment 3 (250 ppm. Delvocid®), Treatment 4 (5000 ppm Propionic Acid), Treatment 5 (5000 ppm. Propionic Acid pressure cooked), Treatment 6 (No inhibitor cooked).

[a]No difference ($p<.05$) between row means.

[b]No difference ($p<.05$) between column means.

TABLE 9. THE EFFECT OF DELVOCID® ON FREE AMONIA[A], ON A AS IS BASE, OF A CORN BASED LIQUID SUPPLEMENT DURING STORAGE

Treatments[B]	Weeks 0	2	4	8	Treatment Average
1 A&B	1.454[a]	1.543[a]	1.563[a]	1.536[a]	1.523[i]
2 A&B	1.736[a]	1.652[a]	1.562[a]	1.574[a]	1.631[i]
3 A&B	1.861[a]	1.972[a]	1.749[b]	1.784[a,b]	1.842[j]
4 A&B	1.663[a]	1.869[b]	1.615[a]	1.623[a]	1.692[j,i]
5 A&B	1.576[a]	1.672[a]	1.718[a]	1.729[a]	1.673[j,i]
6 A&B	2.350[a]	2.649[b]	5.902[c]	6.278[d]	4.295[h]
Weekly Means All Treatments	1.773[a]	1.893[a]	2.351[b]	2.421[b]	
Weekly Means with Treatment 6 Removed	1.658[a]	1.742[a]	1.641[a]	1.650[a]	

[A]Free amonia is expressed as the average of duplicate determination of two replications as mg NH_3-N/g of supplement.

[B]Treatment 1 (150 ppm. Delvocid®), Treatment 2 (200 ppm. Delvocid®), Treatment 3 (250 ppm. Delvocid®), Treatment 4 (5000 ppm. Propionic Acid), Treatment 5 (5000 ppm. Propionic Acid pressure cooked), Treatment 6 (no inhibitor cooked).

[a,b]Row means with the same superscript (a,b) are not different ($p<.05$).

[h,i]Column means with the same superscript (h,i) are not different ($p<.05$).

TABLE 10. THE EFFECT OF DELVOCID® ON FREE AMONIA[A] ON A DRY BATTER BASES, OF A CORN BASED LIQUID SUPPLEMENT DURING STORAGE

	WEEKS				
Treatments	0	2	4	8	Treatment Average
1 A&B	3.44[a]	3.628[a]	3.751[a]	3.800[a]	3.656[h]
2 A&B	4.144[a]	3.943[a]	3.774[a]	3.810[a]	3.920[h,j]
3 A&B	4.441[a]	4.754[a]	4.210[a]	4.235[a]	4.410[i]
4 A&B	3.981[a]	4.489[a]	3.869[a]	3.904[a]	4.061[i,j]
5 A&B	3.762[a]	3.959[a]	4.110[a]	4.175[a]	4.001[i,j]
6 A&B	5.596[a]	6.289[a]	13.983[b]	15.041[c]	10.223[k]
Weekly Means	4.228[a]	4.510[a]	5.616[b]	5.829[b]	
Weekly Means with Treatment 6 Removed	3.954[a]	4.155[a]	3.943[a]	3.986[a]	

[A] Free amonia is expressed as mg HN_3-N/g dry matter and is the average of duplicates of two replications.

[B] Treatment 1 (150 ppm. Delvocid®, Treatment 2 (200 ppm. Delvocid®), Treatment 3 (250 ppm. Delvocid®), Treatment 4 (5000 ppm. Propionic Acid), Treatment 5 (5000 ppm. Propionic Acid pressure cooked), Treatment 6 (no inhibitor cooked).

[a] Row means with the same superscripts are (a, b) not different ($p < .05$).

[b] Column means with the same superscripts (h, i) are not different ($p < .05$).

TABLE 11. THE EFFECT OF STORAGE ON THE VISCOSITY[A] OF A CORN BASED LIQUID SUPPLEMENT

Treatments	Week 0	2	4	8	Treatment Average
1 A&B	774.0[a]	806.0[b]	783.0[b]	796.0[b]	789.0[h]
2 A&B	754.0[a]	808.0[b]	729.0[a]	778.0[a,b]	767.0[i]
3 A&B	762.0[a]	800.0[b]	766.0[a]	790.0[a]	779.0[h]
4 A&B	750.0[a,b]	770.0[a]	735.0[a,b]	757.0[a]	753.0[i]
5 A&B	748.0[a]	777.0[a,b]	812.0[b]	797.0[b]	783.0[h]
6 A&B	757.0[a]	807.0[b]	797.0[b]	798.0[b]	789.0[h]
Weekly Means	757.16[a]	794.33[b]	770.50[a]	785.67[b]	

[A]Viscosity is reported as averages of duplicate determination of two replicates in cps units. (eps)

[B]Treatment 1 (150 ppm. Delvocid®), Treatment 2 (200 ppm. Delvocid®), Treatment 3 (250 ppm. Delvocid®), Treatment 4 (5000 ppm. Propionic Acid), Treatment 5 (5000 ppm. Propionic Acid pressure cooked), Treatment 6 (no inhibitor cooked).

[a,b]Row means with the same superscript are not different ($p<.05$).

[h,i]Column means with the same superscripts (h,i) are not different ($p<.05$).

TABLE 12. THE EFFECT OF DELVOCID® ON *IN VITRO* PROTEIN SYNTHESIS[A] OF A CORN BASED LIQUID SUPPLEMENT DURING STORAGE USING TREATMENT 5 AS A CONTROL

Treatments	Week of Storage 0	2	4	8	Treatment Average
1 A&B	92.0[a]	111.5	97.0	84.3	96.2
2 A&B	91.85	94.5	107.0	92.0	96.3
3 A&B	96.8	108.5	93.3	93.5	98.0
4 A&B	98.0	115.3	105.5	83.8	100.6
5 A&B	Control	Control	Control	Control	Control
6 A&B	97.75	106.75	95.25	98.00	99.4

[A]Protein synthesis is reported as the average of 2 runs and replications during each week of storage on a dry matter protein basis and has been corrected to a percentage of treatment 5.

[B]Treatment 1 (150 ppm. Delvocid®), Treatment 2 (200 ppm. Delvocid®), Treatment 3 (250 ppm. Delvocid®), Treatment 4 (5000 ppm. Propionic Acid), Treatment 5 (5000 ppm. Propionic Acid pressure cooked), Treatment 6 (no inhibitor cooked).

[a]No difference ($p < .05$) in row or column means.

TABLE 13. THE EFFECT OF DELVOCID® ON *IN VITRO* PROTEIN SYNTHESIS[A] OF A CORN BASED LIQUID SUPPLEMENT DURING STORAGE USING TREATMENT 6 AS A CONTROL

Treatments	WEEK OF STORAGE 0	2	4	8	Treatment Average
1 A&B	96.6[a]	106.2	101.8	87.85	98.1
2 A&B	95.00	90.8	112.8	98.3	99.2
3 A&B	99.5	103.50	96.00	98.8	99.4
4 A&B	100.8	109.3	112.8	86.8	102.3
5 A&B	103.0	96.00	107.3	101.8	102.0
6 A&B	Control	Control	Control	Control	Control

[A]Protein synthesis is reported as the average of 2 runs on two replicates during storage on a dry matter protein basis and has been corrected to a percentage of Treatment 6.

[B]Treatment 1 (150 ppm. Delvocid®), Treatment 2 (200 ppm. Delvocid®), Treatment 3 (250 ppm. Delvocid®), Treatment 4 (5000 ppm. Propionic Acid), Treatment 5 (5000 ppm. Propionic Acid pressure cooked), Treatment 6 (no inhibitor cooked).

[a]No difference ($p < .05$) in row or column means.

THE EFFECT OF STORAGE ON THE ACTIVITY OF MONENSIN, VITAMIN A AND TRACE MINERAL CONCENTRATION IN A CORN BASES LIQUID SUPPLEMENT

Introduction

An increasing interest in the use of liquid supplements to supply protein to grazing cattle has prompted this research. Na-Monensin a biologically active compound produced by a strain of Streptomyces cinnamonensis has been shown to alter the production of propionate during ruminant dermentation, Richardson et al (1974), Potter et al (1974), and Utely (1976). This compound has been approved for use in feedlofts and in grazing situations but not for use in liquid supplements due to instability at pHs below 4.2. Also problems with the suspendability of this material throughout the liquid supplement has been questioned. In the preparation of Monensin sodium, monensin is exposed to sodium ions during a pH adjustment and the result is monensin sodium. With this in mind and work done by Choy et al (1974), Estrada-O et al (1972), & Cassler (1971) on the ability of monensin to actively work against pH gradients in transportation of monvalent ions across membranes. It was felt by this author that an increase in sodium ion concentration along with the buffering capacity of the rumen (between pH 5.6 & 6.7) could stabilize Monensin and thus lead to better recovery from a liquid supplement stored at a pH below 4.2. A pH of 3.6 was adopted for this study. In this way other problems arising from feeding liquid supplements can be approached (i.e. over consumption, and ammonia toxicity,

Wiggin and Augustine (1958)).

Vitamin A a water soluble vitamin required by the ruminant also poses a problem when supplied in a liquid supplement due to its low stability when subject to ultraviolet light and oxygen, and due to its suspendability in a liquid media. For this reason a vitamin A palmitate incapsulation in gelatin, described in literature from the Pfizer Co., was used in this study. Also the effect of two different types of trace mineral supplements were evaluated for two criteria: (1) the suspendability in the liquid supplement and (2) their effects upon vitamin A and Monensin activity.

MATERIALS AND METHODS

Hydrothermal Processing:

The liquid supplement used in these studies was processed through a modified Penick and Ford[1] laboratory continuous jet cooker. This cooker was equipped with a Moyno pump that was capable of delivering 1.7 liters of slurry to the hydroheater per minute. Live steam was injected into the slurry through the Hydroheater[R] allowing for a temperature of 160°C and pressure of 55 psig to be achieved. This temperature and pressure is regulated by the use of a steam inlet valve. The temperature and pressure along with the retention time in the cooker causes extensive shearing and cooking of the contents of the liquid supplement.

[1]Penick and Ford, Ltd. Cedar Rapids, Iowa.

Processing of Liquid Supplement Samples:

Table I shows the formulas of the 1:1 (corn to urea on a dry matter basis) 60% protein equivalent liquid supplement. Enough corn, urea, and water (before processing) to make 150 pounds of final product were mixed in stainless tubs. Freatment numbers 2 and 4 had the monensin, trace mineral sources, and vitamin A added after cooking where as 1 and 3 had only the vitamin A added after processing, the remaining ingredients were added to the slurry before processing.

Once the slurries were prepared they were introduced into the reservoir of the jet cooker apperature (Figure I). From this reservoir the slurry was conveyed to the hydroheater by way of a mono pump that delivered 1.7 liters of slurry per minute. At the Hydroheater^{R} live steam is injected into the slurry under pressure. This pressure and heat causes shearing and rupturing of the starch granules of the corn. The temperature and pressure during processing was held constant at 150° and 55 psig. The water absorbed, given in Table 1, was due to the condensation of steam during the cooking process. The processed slurry was collected in an insulated 208 liter steel drum that was equipped with a stirring mechanism. After the entire sample was processed α-amylase was added at a rate of 0.12% of the starch content of the supplement. This enzyme was allowed to react for 14 minutes at a constant temperature of 70° at which time the enzyme activity was stopped through the addition of 1% phosphoric acid.

The product was then cooled to 45°C and the pH adjusted to a pH of 3.7 by the addition of phosphoric acid. At this time the remaining ingredients were added to each treatment.

Each treatment was produced in duplicate and introduced into storage vessels chosen at random to eliminate differences in storage containers. These storage containers were made from 6 inch PVC plastic pipe that was cut into 5 foot sections and sealed at one end. Sampling holes were introduced into the pipe at one foot increments and in the middle of the bottom. This allowed for sampling of the supplements at five locations. Approximately 50 g samples of each supplement treatment were taken at 0,2,4,6, & 8 weeks during the trial. These samples were used to determine the chemical and suspendable stability of monensin, vitamin A, and the trace minerals zinc, magnesium, and iron. A composit sample of each replication was used to determine the stability of this supplement.

Monensin Chemical Stability:

The stability of this antibiotic was determined through the modification of the vanillin colorimetric method developed by Golab et al (1973), in which 10 grams of supplement was mixed with 20 ml of buffer which contained electrolytes at a concentration similiar to saliva. This mixture was shaken for one hour at medium speed on a Eberbach[1] rotating shaker. At the end of one hour 100 ml of acetone; etheyl ether

[1]Eberbach Corporation, Ann Arbor, Mich.

(50:50) was added to the flask and shaken for one hour on the Everbach rotating shaker and then allowed to stand overnight. A mixture of acetone and ether was used to eliminate dehydration of the sample. Dehydration was found to decrease extraction of the monensin into the acetone phase. After setting overnight 6 ml of the top phase (acetone:ether) was transferred to a 50 ml beaker and carefully evaporated. Preliminary studies showed no monensin to be present in the lower phase of this two phase system. This agreed with the data monensin is not soluble to any significant degree in water systems, Galab et al (1973).

The contents of the 50 ml beaker was transferred to a 10 ml volumetric flask and diluted to volume with methonol. Five milliliters of this dilution was transferred to a second ten milliliter volumetric flask that had been spiked with 30 ug of monensin[1] standard. To each of these flasks 1.0 ml of vanillin reagent was added and the flask diluted to 10 ml with methanol. The absorbance of each solution was read at 518nm against a methanol blank and poured back into the flask. The flask was then heated at 60°C for 25 minutes and cooled and absorbance was then determined The calcultion of monensin concentration in the original sample was determined by the following formula:

$$(\text{Absorbace heated sample} - \text{Absorbance unheated}) \times \frac{20\text{ml}}{10\text{ g}} \times$$

$$\frac{100\text{ml}}{20\text{ml}} \times \frac{10\text{ml}}{6\text{ml}} \times \frac{10\text{ml}}{5\text{ml}} \times \frac{30\text{ug}}{10\text{ml}} \times \frac{1\text{g}}{10^6\text{ug}} \times \frac{2000\text{lb}}{\text{ton}} \times \frac{454\text{g}}{1\text{b}} =$$

Monensin, g/ton.

[1]Elli Lyle Inc. Greenleaf, Ind.

Duplicates of each sample were determined and results are given in Table 1 of Appendix B.

Vitamin A Chemical Stability Determination:

The chemical activity of Vitamin A was determined by using the direct solvent extraction technique of Bayfield (1971). In this procedure one gram of the supplement was added to 50 ml of a 1:1 mixture of acetone and light petroleum ether. This mixture was then shaken for 30 minutes on the Eberbach rotating shaker and allowed to stand for 30 minutes in order that the particulate matter could settle from the extracting liquid. A 5 ml alequot of the extraction fluid was evaporated to dryness and dissolved in 5 ml of chloroform. One milliliter of the chloroform mixture and 2.0 ml of saturated trichloroacetic acid was added to a spectra grade cavet. The absorbance of this mixture was recorded at 620 nm and concentration on vitamin A determined by extrapolation from a standard curve. Duplicate determination of each sample was made and results are given in Table 2 of Appendix B.

Trace Minerals: The concentration of zinc, iron, and magnesium were determined by Atomic Absorption analysis as follows: A 5 - 10 gram sample of the corn base supplement was accurately weighed and dried at 50°C overnight. The residue remaining after this drying step was ashed at 550° overnight. Enough 6 N HCl was added to the ashed sample to dissolve it.

This solution was then quantitatively transferred to a 25 ml volumetric flask and diluted to the mark with dionized distilled water. The diluted sample was then asperated into the air-acetylene flame of a Jarrel Ash Atomic Absorption Spectrophotometer. The concentration of the three minerals was determined at the following wavelengths; iron 2482Å, zinc 2138Å, and magnesium 2852Å. The concentration of Zinc and Iron were determined directly from Beers Law standard curve, however the magnesium concentration was determined by diluting the original sample 1 to 100 with dionized distilled water. Averages of duplicate determinations of each sample are reported in ppm on a dry basis in Table X of Appendix A.

pH

The pH of the composite samples of each replication was determined on a Beckman pH meter to the nearest hundreth of a pH unit.

Statistical Analysis:

SAS 76 is a canned statistical analysis program that was developed by Barr et al (1976 which was used to analize the data collected for the above mentioned dependent variables. Differences between treatment means, weekly means, level means, and interactions was determined by use of a Split-Split Plot design. The treatments were analyzed as the whole plots, with the week storage analyzed as the split-plots and the level of storage analyzed as the split-split plots. Least significant difference (LSD) analysis was used to determine differences

in the means investigated. The statistical analysis of variance table (AOV) are found in Appendix D.

Results and Discussion:

The objectives of this study were to determine (1) the effect of NaCl addition on the stability of Monensin chemical activity; (2) to determine the effect of storage on this chemical stability; (3) to determine the stability of vitamin A palmatate activity during storage; (4) to determine the ability of two soluble trace mineral supplements to remain suspended during 8 weeks of storage; and (5) to determine the effect these trace minerals had on the chemical activity of monensin and the activity of vitamin A.

Effect of NaCl addition on Monensin Chemical Activity:

A 100 lb batch of hydrothermally processed liquid supplement was subdivided into 10-10 lb subunits and Rumensin 30^{R} (30 mg of Monensin) was added to each to give a final Na-Monensin concentration of 200 mg/lb of supplement. Table 2 lists the amount of NaCl added to each of 5 samples, the remaining 5 samples were used as replications of these treatments. The chemical activity of Na-Monensin was determined as reported earlier by the Modified Vanillin colorimetric procedure. Table 3 shows the results of this study. It can be seen that the addition of .5% NaCl gave a recovery of 90% of the initial concentration of Na-Monensin and the addition of 2,5, & 10% NaCl increased this recovery considerable. From the results of this study treatment of 5% NaCl was selected

to use in an eight week storage study to determine whether or not the chemical activity of Na-Monensin would remain above 90% of the original.

Chemical Activity of Monensin During Storage:

Table 4 shows the treatment averages of the five sampling levels during each week of storage. The analysis of variance, Table 12 Appendix D, table for this dependent variable. There were no difference ($p<.05$) found in treatment and level means, how ever a difference was found in the weekly averages. A LSD test ($p<.05$) (least significant difference), Table 5, however showed there was no difference in treatment means, week of storage means, or level of storage means. One needs to look at the interaction to determine where differences existed. From the analysis of variance, Table 12 Appendix D, it is seen that the week by treatment, treatment by level, and treatment by level by week were the significant interactions. Table 6 lists the means for weekly determination for each treatment. A LSD ($p<.05$) has shown no difference to exist in treatment 1 during storage, where was a difference ($p<.05$) found in treatment 2 in the 4th week of storage. This was an increase in activity and therefore was not a detrimental effect. This may be due in part to a sampling error during this weeks determination since level 2 of replication 1 and levels 2 and 3 of replication 2 for this treatment had exceptionally high activity during the 4th week of storage. This activity of Na-Monensin in this treatment returned to the original level during the 6th and the 8th week of storage which conforms the

idea of sampling error during week 4. In treatments 3 and 4 there was a difference ($p<.05$) found in the 2nd week of storage and again this was an increase in activity from the original and is believed to be due to sampling error. When these two cases are removed from the study it is seen that there was no difference ($p<.05$) in Na-Monensin activity for any of the treatments during storage.

This then allows one to compare the level of storage means for each treatment during storage. Table VII lists these means and their respective LSD ($p<.05$). There was no difference ($p<.05$) found between any level sampled during storage. Thus there was no effect due to trace mineral source used in this study. This may be extended a step further; there is no stable complex formed between the ionized minerals used that is stronger than the Monensin complex.

Table 7 gives the level of storage means for monensin acttivity and Table 8 the treatment by week combination. All levels of storage sampled for all treatments were the same at the beginning of the trial, ranging from 398 to 404 mg/lb of supplement. At week 2 of storage all treatments showed a tendency of segregation towards the top and center of the storage tank. Week 4 showed the same trend as week 2. After 6 weeks of storage however there was no difference between levels in treatments 1,3, and 4. Treatment 2 showed difference ($p<.05$) however there was a shift back towards equilibrium in the tank. At the end of the trial (week 8) there were no differences ($p<.05$) found in any of the treatments.

From these results it may be concluded that Na-Monensin remained suspended well in the corn based liquid supplement

during the entire eight weeks of storage.

As for the chemical activity of Monensin it remained the same throughout the trial even at a constant pH of 3.6 as reported in Table 9.

Further work must still be done to determine if the biological activity as well as the chemical activity is maintained when the supplement is stored and fed at a pH of 3.6 and treated with 5 to 10% added NaCl.

Vitamin A Stability:

Table 13 Appendix D, gives the analysis of variance for the vitamin A data collected during this 8 week trial. Of the main effect treatment, week of storage, and level of storage only the week of storage was different ($p<.05$). Table 10 gives the weekly averages for the trial. The initial activity of vitamin A was 130 ug/g or 107,309 IU of vitamin A per pound. There was no difference ($p<.05$) found in activity after the 2nd week of storage (130.09 as compared to 131.07 ug/g). After 4 weeks of storage however a difference ($p<.05$) was found and this difference increased after the 6th week of storage and then remained the same through 8 weeks of storage. There was a decrease of 4,820 IU of Vitamin A activity during the 4th week and a 8,353 IU & 8,114 IU decrease from the initial activity at week 6 and 8 of the trial respectively. This decrease in activity may be due to the oxidation of the vitamin by ultraviolet light and/or due to oxidation caused by the interaction between the trace minerals and vitamin A. There was no difference ($p<.05$) between treatment means and level means during storage as shown in Table 10. To determine if

there was an effect of trace mineral supplement used on the activity of vitamin A one must look at the interaction between the main effects.

In the analysis of variance Table 13 Appendix D, it is seen that the only difference ($p<.05$) interaction is between weeks of storage and treatment. Table 11 gives the week of storage means for each treatment. As LSD ($p<.05$) has shown that there was a difference ($p<.05$) has shown that there was a difference ($p<.05$) in treatments 1 (CTM soluble trace minerals before processing) and in treatment 2 (CTM soluble trace minerals added after processing). This difference ($p<.05$) is seen in the 4th, 6th, and 8th week of storage. During the 4th week there was no difference ($p<.05$) however there was a decrease in activity from 130 ug vitamin A palmitate/g of supplement to 125 ug of vitamin A palmitate in treatment 1 and a decrease from 130 to 120 in treatment 2. This decrease in activity became significant ($p<.05$) after 6 weeks of storage. Treatment 1 had only 117 ug of activity or 90% of the initial no further loss of vitamin A after 8 weeks of storage.

In treatments 3 and 7 (Sea-Questra Min trace mineral, added before and after processing respectively), there were no difference ($p<.05$) between the beginning and the end of the eight week storage study. Thus there was a difference due to the type of trace mineral used in conjunction with Vitamin A palmitate in this corn based liquid supplement. This is supported by work done by the Stauffer Chemical Company (1976) in which Sea-Questra Min was compared to other

trace mineral supplements to determine what effect it had on vitamin A activity when used in dry and molasses based premixes. However with only 10% loss in activity it is felt that either trace mineral supplement may be used with vitamin A.

Comparison of Trace Mineral Supplement Suspensibility:

To evaluate the suspensibility of the minerals present in the two supplements used in this study samples from levels 1 (top), 3 (middle), and 5 (bottom) were analyzed by the atomic absorption method previously described. Table 14 of Appendix D, gives the analysis of variance for the concentration of iron, magnesium, and zinc determined in this trial. From this analysis it is seen that the main effect due to treatment is the only significant effect ($p<.05$) for the iron concentration during storage. The main effect due to treatment, and week of storage are significant ($p<.05$) for the magnesium concentration. Also the interactions between week of storage and treatment is significant ($p<.05$) for this mineral. There was no significant ($p<.05$) effects for thw zinc concentration found in this study.

Table 12 gives the means treatments, week of storage, and the level of storage for all three minerals investigated. From the LSD ($p<.05$) it is seen that the difference found in the effect due to treatment for iron concentration is only found in treatment 4. This can partially be explained by the low levels of iron found in the top and middle levels of storage for treatment 4 replication 2 during the 2nd, 4th, and 6th weeks of storage, as reported in Table 1 of Appendix C. This is

probably do to error in sampling these two levels when the analysis was run. Therefore comparison between iron concentrations for level of storage means can only be made between the levels in which the week of storage by treatment means are not different ($p<.05$). Table 13 lists the means for the week of storage by treatment combinations. An LSD ($p<.05$) found in treatment 1 & 4 for all weeks of storage. Treatments 2 & 3 showed a difference and therefore will not be used to examine the suspensibility of this mineral.

Table 14 shows the level means for treatment 1 (CTM soluble) and treatment 4 (Sea-Questra-Min) and compares the concentration of each weekly level to the original concentration at that level as a percentage of week 0. It is seen from this table that the iron concentration shifted towards the top of the storage vessels in both sources of minerals, with the CTM soluble iron migrating more towards the top than the Sea-Questra-Min (CTM; top level 122%, middle level 100%, and bottom level 85% as compared to Sea-Questra-Min; top 113%, middle 91%, and bottom 95%). Thus the claim made by the Stauffer Chemical Company that sequestered trace minerals stay in suspension better than chealated minerals holds true in the case of iron for this liquid supplement.

Table 10 shows that there was a difference ($p<.05$) in magnesium concentration between treatments 1 & 2 and treatments 3 & 4 this is due to the difference in percent composition of the two trace mineral sources. However a comparison may still be made between the sources. Table 15 gives the treatments means at each week of storage. The LSD ($p<.05$) shows no difference in means for the first 6 weeks of storage for

treatments 1 & 2 and no difference in means for the first 4 weeks of storage for treatments 3 & 4. Therefore comparison may be made on level magnesium concentration for the first 4 weeks of storage. Differences in the other weekly means are due to sampling error as shown in the replicate data reported in Table II of Appendix C.

Table 16 gives the lever of storage means for the first 4 weeks of storage for each treatment reported as a percent of week 0. During the first 2 weeks of storage there was a tendency for the magnesium to settle to the bottom of the tank in treatment 1 (CTM added before) and treatment 3 (Sea-Questra-Min added before). In treatment 2 (CTM added after) and treatment 4 (Sea-Questra-Min added after) there was not much change in the level of concentrations of magnesium in the first 2 weeks of storage. After 4 weeks of storage all treatments maintained essentially the same magnesium level as week zero. Therefore it can be seen that magnesium concentration after 4 weeks is dependent of source used.

The zinc concentration during storage was independent of treatment since there was no difference ($p<.05$) found in mean treatment concentration as reported in Table 10. Table 17 lists the zinc concentrations by week of storage and it can be seen that the only two treatments in which there is no difference ($p<.05$) are treatment 1 (CTM added before) and treatment 4 (CTM added after) the other two treatments have too large of differences between weekly means to allow for comparison. Therefore Table 18 lists the mean level concentration of zinc for treatments 1 & 4 expressed as a percent of week 0. Again as in the case of iron concentra-

tion the zinc contration remained constant throughout storage when the source of trace minerals was Sea-Questra-Min.

The results of this study leads one to believe that Sea-Questra-Min in the trace mineral supplement of choice for this liquid supplement. However due to the large deviation found in the weekly means of treatments it is felt that this conclusion can not be made without reservation.

SUMMARY

Monensin chemical activity, Vitamin A palmitate activity, and trace mineral suspendibility were investigated in a corn based liquid supplement. It was found that when Na-Monensin was added to this supplement at a pH of 3.6 there was basically no chemical activity. However the addition of NaCl at a level of either 5 or 10% caused a change in this feed additive's stability. An 8 week storage study showed that at the level of 5% added NaCl the chemical activity of Monensin was maintained at 400 mg/lb of supplement. It was also found that there was no effect on activity when trace mineral supplements were added.

It was found that Vitamin A activity was affected by the type of trace mineral supplement. The activity of this vitamin remained the same ($p<.05$) when the sequestered trace minerals were used. The activity decreased after 4 weeks of storage when chealated trace minerals were used (130 ug to 122 ug). This decrease continued during the 6th week of storage (117ug) and then remained the same at 8 weeks of storage. However the loss in activity of Vitamin A was only 10% over the 8 weeks of storage and there was a loss of 5% of the activity due to ultraviolet light oxidation.

When looking at the suspendibility of the trace mineral sources, it was found that the sequestered source of iron and zinc remained constant throughout the trial. The chealated source however migrated to the top of the tank. Magnesium concentration however changed in both sources of trace

minerals. This change was towards the bottom of the tank.

From the results of this study it can be concluded that Monensin remains chemically active in liquids stored and fed at a pH of 3.6 when 5% added NaCl is used. Also Vitamin A activity and trace mineral concentration remained constant when a sequestered trace mineral source is used.

TABLE 1. FORMULATIONS OF 60% PROTEIN EQUIVELANT CORN BASED LIQUID SUPPLEMENT

% Ingredient	TRT 1	TRT 2	TRT 3	TRT 4
Corn	23.1	23.1	23.1	23.1
Urea	20.8	20.8	20.8	20.8
H_2O Before Cooking	39.00	39.00	39.00	39.00
H_2O Absorbed During Processing	10.35	10.35	10.35	10.35
NaCl	5.00	5.00	5.00	5.00
Rumensin 30[1]	.67	.67	.67	.67
Sea-Questra Min[2]	-	-	.20	.20
CTM Soluble[3]	.20	.20	-	-
Vitamin A (Pfizer A 250 m [4])	.088	.088	.088	.088
Phosphoric Acid (75% Food Graded)	1.5	1.5	1.5	1.5
Proprionic Acid	.5	.5	.5	.5
Total	100.008	100.008	100.008	100.008

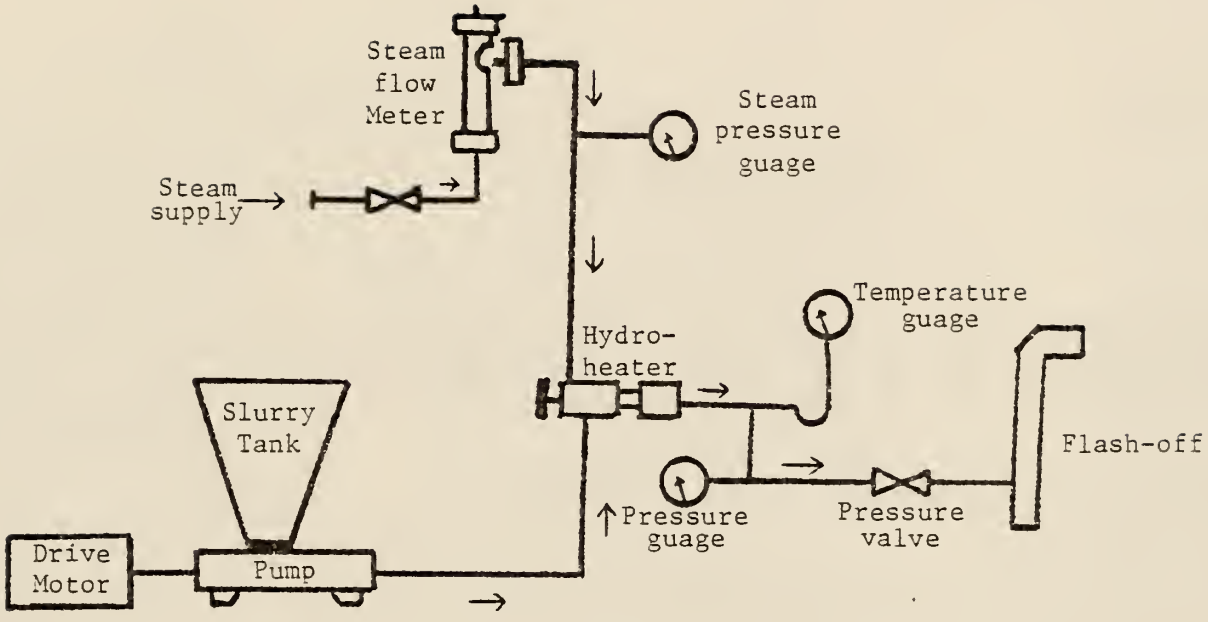
[1]Elano Products Company, Indianapolis, Indiana

[2]Stauffer Chemical Company, Westport, Connecticut

[3]Arizona Feeds, Tucson, Arizona

[4]Chas. Pfizerer & Co., New York, New York

FIGURE I



Schematic layout of the Hydrothermal Jet Cooker.

TABLE 2. COMPOSITION OF TREATMENT USED TO DETERMINE THE EFFECT OF NaCL ADDITION TO A CORN BASED LIQUID SUPPLEMENT

TRT	Corn Based Liquid Supplement	Rumensin 30 lb.	Calculated mg/lb/Supplement	NaCl lb.	% of Supplement
1	49.666	.334	200	0	0
2	49.166	.334	200	.5	.5
3	47.666	.334	200	2	2
4	44.666	.334	200	5	5
5	39.666	.334	200	10	10

TABLE 3. DETERMINED CONCENTRATION OF MONENSIN SODIUM AFTER ONE DAY OF STORAGE

TRT	Momensin Sodium[A] mg/lb of Supplement	% of Calculated	% NaCl
1	24.24	12.12	0
2	180.0	90.0	.5
3	190.0	95.0	2
4	196.0	98.0	5
5	198.0	99	10

[A] Na-Monensin is reported as averages of duplicate runs of each replication.

TABLE 4. THE EFFECT OF 5% NaCL ADDITION ON THE CHEMICAL ACTIVITY OF MONENSIN-SODIUM[A] DURING STORAGE

Sample[B] TRT	Level	Week of Storage 0	2	4	6	8	Average Level Sampled
1	1	399.0	395.8	401.9	401.9	399.6	399.6
1	2	399.0	409.4	398.2	396.6	398.2	400.2
1	3	399.0	406.8	405.7	403.3	400.5	403.3
1	4	399.0	397.7	398.2	398.9	398.8	398.5
1	5	400.0	396.8	403.6	398.1	400.1	399.7
2	1	400.0	408.9	400.9	392.1	400.0	400.4
2	2	399.5	395.8	417.0	398.5	398.8	401.9
2	3	397.5	408.5	408.1	400.7	399.4	402.8
2	4	401.0	404.2	405.1	400.5	400.5	402.3
2	5	401.0	402.5	406.7	400.5	399.3	402.0
3	1	398.5	407.3	404.1	400.9	401.4	402.4
3	2	398.0	408.5	396.4	396.0	399.9	399.8
3	3	403.5	395.8	396.4	397.8	397.9	398.3
3	4	400.5	411.3	388.3	390.1	400.3	398.1
3	5	402.0	399.4	405.6	400.9	398.6	401.3
4	1	399.0	403.0	396.8	394.2	400.2	398.6
4	2	399.0	407.3	396.3	394.6	400.6	399.6
4	3	400.5	465.0	390.0	393.8	398.8	397.6
4	4	399.5	408.3	397.4	398.8	398.9	400.6
4	5	400.0	400.6	394.8	397.2	395.5	397.6

[A]Monensin-Sodium chemical activity reported as mg/lb and are averages of duplicates.

[B]Treatment 1 (CTM and Monensin-Sodium added before), Treatment 2 (CTM and Monensin-Sodium added after), Treatment 3 (Sea-Questra-Min and Minensin-Sodium added before), Treatment 4 (Sea-Questra-Min and Monensin-Sodium added after).

TABLE 5. THE EFFECT OF STORAGE ON TREATMENT, WEEK, AND LEVEL MEANS

Treatment Means

	TRT[A]			
Means	1	2	3	4
	400	402	400	399

Weekly Means

	Week of Storage				
Means	0	2	4	6	8
	400	404	401	398	399

Level Means

	Level[A] of Storage				
Means	1	2	3	4	5
	400	400	400	400	400

[A] No significant differences ($p < .05$).

TABLE 6. WEEKLY NA-MONENSIN CHEMICAL ACTIVITY FOR EACH TREATMENT

TRT[A,B,C]	Week[A,B] of Storage 0	2	4	6	8
1	399 a,h	401 a,h	402 a,h,i,	400 a,h	399 a,h
2	400 a,h	404 a,b,h	408 b,h	398 a,h	400 a,h
3	401 a,b,h	404 a,h	398 b,i,j	397 b,h	400 a,b,h
4	400 a,b,h	405 a,h	395 b,i,j	396 b,h	398 b,h

[A] Row means with the same superscript (a,b) are not different ($p<.05$).

LSD = 5

[B] Column means with the same superscript (h,i) are not different ($p<.05$)

LSD = 6

[C] Treatment 1 (CTM and Monensin-Sodium added before), Treatment 2 (CTM and Monensin-Sodium added after), Treatment 3 (Sea-Questra-Min and Minensin-Sodium added before), Treatment 4 (Sea-Questra-Min and Monensin-Sodium added after).

TABLE 7. MEAN MONENSIN-SODIUM[A] CHEMICAL ACTIVITY FOR LEVELS WITHIN EACH TREATMENT

TRT[C]	Level[B] 1	2	3	4	5
1	400[a]	400[a]	403[a]	399[a]	400[a]
2	400[a]	402[a]	402[a]	402[a]	402[a]
3	402[a]	399[a]	398[a]	398[a]	401[a]
4	399[a]	400[a]	399[a]	400[a]	398[a]

[A] Na-Monensin is expressed as mg/lb of supplement.

[B] Row means with the same superscript (a,b) are not different($p<.05$)

LSD = 4

[C] Treatment 1 (CTM and Monensin-Sodium added before), Treatment 2 (CTM and Monensin-Sodium added after), Treatment 3 (Sea-Questra-Min and Monensin-Sodium added before), Treatment 4 (Sea-Questra-Min and Monensin-Sodium added after).

TABLE 8. MEAN MONENSIN-SODIUM[A] CHEMICAL ACTIVITY FOR LEVELS WITHIN THE SAME TREATMENT AND WEEK COMBINATIONS

TRT[C]	Week	Level[B] 1	2	3	4	5
1	0	399^{a}	399^{a}	399^{a}	399^{a}	400^{a}
	2	395^{a}	409^{b}	407^{b}	398^{a}	397^{a}
	4	401^{a}	398^{a}	407^{b}	398^{a}	404^{a}
	6	401^{a}	397^{a}	403^{a}	399^{a}	398^{a}
	8	399^{a}	398^{a}	401^{a}	399^{a}	400^{a}
2	0	400^{a}	400^{a}	398^{a}	401^{a}	401^{a}
	2	409^{a}	396^{b}	409^{a}	404^{a}	403^{a}
	4	401^{a}	417^{b}	408^{a}	405^{a}	408^{a}
	6	392^{a}	$399^{a,b}$	401^{b}	401^{b}	$400^{a,b}$
	8	400^{a}	399^{a}	399^{a}	401^{a}	399^{a}
3	0	398^{a}	398^{a}	404^{a}	400^{a}	402^{a}
	2	$407^{a,b}$	$408^{a,b}$	415^{b}	411^{b}	399^{a}
	4	404^{a}	$396^{a,b}$	$396^{a,b}$	388^{b}	406^{c}
	6	400^{a}	396^{a}	398^{a}	390^{a}	401^{a}
	8	401^{a}	400^{a}	398^{a}	400^{a}	399^{a}
4	0	399^{a}	399^{a}	401^{a}	400^{a}	400^{a}
	2	403^{a}	407^{a}	405^{a}	408^{a}	401^{a}
	4	397^{a}	396^{a}	390^{a}	397^{a}	395^{a}
	6	394^{a}	394^{a}	393^{a}	399^{a}	397^{a}
	8	400^{a}	400^{a}	399^{a}	399^{a}	396^{a}

A Na-Monensin chemical activity is expressed in mg/lb of supplement.

B Row means with the same superscripts (a,b) are not different ($p < .05$) LSD = 7.78.

C Treatment 1 (CTM and Monensin-Sodium added before), Treatment 2 (CTM and Monensin-Sodium added after), Treatment 3 (Sea-Questra-Min and Monensin-Sodium added before), Treatment 4 (Sea-Questra-Min and Monensin-Sodium added after).

TABLE 9. pH[A] OF THE CORN BASED LIQUID SUPPLEMENT DURING STORAGE

TRT[B]	0	2	4	6	8
1	3.60	3.61	3.60	3.60	3.60
2	3.60	3.60	3.61	3.61	3.60
3	3.60	3.62	3.60	3.60	3.60
4	3.60	3.60	3.62	3.61	3.60

[A] There was no difference ($p < .05$) in the pH values of treatments during storage.

[B] Treatment 1 (CTM and Monensin-Sodium added before), Treatment 2 (CTM and Monensin-Sodium added after), Treatment 3 (Sea-Questra-Min and Monensin-Sodium added before), Treatment 4 (Sea-Questra-Min and Monensin-Sodium added after).

TABLE 10. WEEK OF STORAGE, TREATMENT AND LEVEL OF STORAGE MEANS VITAMIN A[A] ACTIVITY

Mean[B]	Week 0	2	4	6	8
	130.09[a]	131.07[a]	124.16[b]	119.88[c]	120.17[c]

[A]Vitamin A activity is expressed in ut Vitamin A palmitate/ g of supplement.

[B]Row means with the same superscript (a,b) are not different (p .05) LSD = 2.84.

Means[B]	Treatment 1	2	3	4
	123.53[a]	124.55[a]	125.61[a]	126.59[a]

Means[B]	Level 1	2	3	4	5
	124.69[a]	125.04[a]	124.77[a]	124.57[a]	126.30[a]

[A]Vitamin A activity is expressed in ug of Vitamin A palmitate/ g of supplement.

[B]Row means with the same superscript (a,b) are not different ($p<.05$) $LSD_{TRT} = 3.06$ $LSD_{Level} = 2.42$.

[C] Treatment 1 (CTM added before Vitamin A added after), Treatment 2 (CTM and Vitamin A added after), Treatment 3 (Sea-Questra-Min added before and Vitamin A added after), Treatment 4 (Sea-Questra-Min and Vitamin A added after).

TABLE 11. WEEKLY MEAN VITAMIN A[A] ACTIVITY FOR EACH TREATMENT

TRT	Week[B] 0	2	4	6	8
1	130.05[a]	131.04[a]	124.63[a,b]	116.64[b]	123.29[b]
2	130.15[a]	131.29[a]	119.98[a,b]	117.61[b]	123.71[b]
3	130.15[a]	130.53[a]	128.80[a]	119.49[a]	119.10[a]
4	130.00[a]	131.40[a]	123.24[a]	125.77[a]	122.56[a]

[A] Vitamin A activity is expressed in ug of vitamin A palmitate/g of supplement.

[B] Row weekly means with the same superscript (a,b) are not different ($p<.05$).

[C] Treatment 1 (CTM added before Vitamin A added after), Treatment 2 (CTM and Vitamin A added after), Treatment 3 (Sea-Questra-Min added before and Vitamin A added after), Treatment 4 (Sea-Qeustra-Min and Vitamin A added after).

TABLE 12. MEAN IRON, MAGNESIUM, AND ZINC CONCENTRATIONS[A] FOR TREATMENT, WEEK OF STORAGE, AND LEVEL OF STORAGE

Mean[B]	Concentration	Treatment[E] 1	2	3	4
	Iron	303^{a}	324^{a}	299^{a}	250^{b}
	Magnesium	670^{a}	632^{b}	582^{c}	562^{c}
	Zinc	245^{a}	242^{a}	242^{a}	238^{a}

Mean[C]	Concentration	Week of Storage 0	2	4	6	8
	Iron	302^{a}	290^{b}	285^{b}	278^{b}	314^{a}
	Magnesium	658^{a}	646^{a}	646^{a}	603^{b}	503^{c}
	Zinc	$241^{a,b}$	225^{b}	$250^{a,b}$	$240^{a,b}$	255^{a}

Mean[D]	Concentration	Top	Middle	Bottom
	Iron	299^{a}	296^{a}	286^{a}
	Magnesium	618^{a}	614^{a}	601^{a}
	Zinc	246^{a}	242^{a}	138^{a}

[A] Concentration of trace minerals are expressed in ppm on a dry matter basis.

[B] Row means with the same superscript (a,b) are not different ($p<.05$). $LSD_{Fe} = 34.19$ $LSD_{Mg} = 27.99$ $LSD_{Zn} = 7.03$

[C] Row means with the same superscript (a,b) are not different ($p<.05$). $LSD_{Fe} = 19.78$ $LSD_{Mg} = 14.69$ $LSD_{Zn} = 26.73$

[D] Row means with the same superscript are not different ($p<.05$). $LSD_{Fe} = 18.7$ $LSD_{Mg} = 32.9$ $LSD_{Zn} = 21.91$

[E] Treatment 1 (CTM added before), Treatment 2 (CTM added after), Treatment 3 (Sea-Questra-Min added before), Treatment 4 (Sea-Questra-Min added after).

TABLE 13. MEAN IRON[A] CONCENTRATION FOR ALL WEEKS OF STORAGE BY TREATMENT COMBINATION

Treatment[C]	0	2	4	6	8
1	307[a]	293[a]	301[a]	304[a]	311[a]
2	342[a]	304[a,b]	343[a]	267[b]	364[a]
3	302[a,b]	290[a,b]	270[b]	302[a,b]	329[a]
4	256[a]	273[a]	225[a]	241[a]	254[a]

[A] Iron concentrations are expressed in ppm on a dry matter basis.

[B] Row means with the same superscript (a,b) are not different ($p < .05$).

LSD = 48.29

[C] Treatment 1 (CTM added before), Treatment 2 (CTM added after), Treatment 3 (Sea-Questra-Min added before), Treatment 4 (Sea-Questra-Min added after).

TABLE 14. WEEKLY LEVEL MEAN IRON[A] CONCENTRATION COMPARED TO WEEK ZERO

Treatment 1 (CTM Soluble)

Level	Week of Storage 0	2	% of Week 0	4	% of Week 0	6	% of Week 0	8	% of Week 0
Top	284	310	109	311	110	220	95	346	122
Middle	318	286	90	286	90	319	100	318	100
Bottom	319	283	89	307	96	323	101	270	85

Treatment 4 (Sea-Questra-Min)

Level	Week of Storage 0	2	% of Week 0	4	% of Week 0	6	% of Week 0	8	% of Week 0
Top	239	324	135	213	89	233	97	270	113
Middle	258	258	100	225	87	239	93	235	91
Bottom	271	236	87	237	87	251	93	256	95

TABLE 15. MEAN MAGNESIUM[A] CONCENTRATION FOR WEEKLY ANALYSIS OF EACH TREATMENT

Treatment[B,C]	Week of Storage 0	2	4	6	8
1	699[a]	678[a]	689[a]	650[a,b]	633[b]
2	673[a]	686[a]	628[a]	653[a]	517[b]
3	658[a]	615[a]	648[a]	556[b]	435[b]
4	603[a]	608[a]	620[a]	552[b]	426[b]

A Magnesium concentration expressed in ppm on a dry matter basis.

B Row means with the same superscript (a,b) are not different ($p<.05$).

LSD = 49.58

C Treatment 1 (CTM added before), Treatment 2 (CTM added after), Treatment 3 (Sea-Questra-Min added before), Treatment 4 (Sea-Questra-Min added after).

TABLE 16. MEAN LEVEL MAGNESIUM[A] CONCENTRATION FOR THE FIRST FOUR WEEKS OF STORAGE

Treatment[B]	Level	Week of Storage 0	2	% of Week 0	4	% of Week 0
1	Top	677	671	99	709	105
	Middle	701	622	89	650	93
	Bottom	719	738	103	708	98
2	Top	681	649	95	674	99
	Middle	660	689	105	581	88
	Bottom	674	720	106	630	93
3	Top	687	532	77	692	101
	Middle	659	606	92	607	92
	Bottom	627	705	112	643	103
4	Top	647	635	98	618	96
	Middle	577	612	106	639	111
	Bottom	584	576	99	602	103

[A] Magnesium concentration is expressed in ppm on a dry matter basis.

[B] Treatment 1 (CTM added before), Treatment 2 (CTM added after), Treatment 3 (Sea-Questra-Min added before), Treatment 4 (Sea-Questra-Min added after).

TABLE 17. MEAN ZINC[A] CONCENTRATION FOR WEEKLY ANALYSIS OF EACH TREATMENT

Treatment[B,C]	Week of Storage 0	2	4	6	8
1	270[a]	236[a,b]	257[a]	227[a]	238[a]
2	239[a]	221[a]	280[b]	205[a]	268[b]
3	220[a]	205[a]	206[a]	297[b]	283[b]
4	236[a]	237[a]	253[a]	235[a]	230[a]

[A] Zinc concentration expressed in ppm on a dry matter basis.

[B] Row means with the same superscripts (a,b) are not different ($p < .05$). LSD = 43.83

[C] Treatment 1 (CTM added before), Treatment 2 (CTM added after), Treatment 3 (Sea-Questra-Min added before), Treatment 4 (Sea-Questra-Min added after).

TABLE 18. MEAN LEVEL ZINC[A] CONCENTRATION FOR TREATMENTS 1 AND 4 DURING STORAGE

Treatment 1 (CTM Soluble Added Before)

Level	Week of Storage 0	2	% of Week 0	4	% of Week 0	6	% of Week 0	8	% of Week 0
Top	261	245	94	270	103	174	67	232	89
Middle	275	203	73	207	75	265	96	269	198
Bottom	273	261	95	294	108	242	88	211	77

Treatment 4 (Sea-Questra-Min Added After)

Level	Week of Storage 0	2	% of Week 0	4	% of Week 0	6	% of Week 0	8	% of Week 0
Top	230	232	100	271	118	210	91	232	101
Middle	231	205	89	255	110	254	110	230	100
Bottom	246	275	112	234	95	242	98	229	93

[A] Zinc concentration expressed in ppm on a dry matter basis.

SUMMARY OF THESIS

Two storage trials were conducted to determine: (1) if the product Delvocid® could be used as a preservative in a corn based liquid supplement; (2) to determine what steps need to be taken to maintain Monensin's chemical activity in a corn based liquid stored at a pH of 3.6; (3) to determine the stability of Vitamin A palmitate in this liquid supplement when two different types of trace minerals were used; (4) to determine the suspensibility of trace mineral from the two sources used.

In study one concentrations of 150, 200, and 250 ppm of Delvocid were compared to a positive control (5,000 ppm propionic acid) and to a negative control (no preservative added). The parameters pH, crude protein, dry matter, ammonia release, maltose equivalence, protein synthesis, and viscosity were monitored during the study. Results indicated that either the 200 or the 250 ppm level of Delvocid was able to maintain constant values during the trial and it compared well with the propionic acid control.

Preliminary investigation on the effect of NaCl addition on Na-Monensin chemical activity indicated that a level of 5 or 10% was capable of maintaining activity for 24 hours. To investigate the effect of 5% NaCl to maintain chemical activity during storage and to determine the stability of Vitamin A palmitate and the effects of trace mineral supplements on these feed additives, a second storage trial was run. The results of this study indicated that Na-Monensin's chemical activity was maintained during 8 weeks

of storage. There was no difference ($p<.05$) in the chemical activity when trace mineral sources were compared. The activity of vitamin A palmitate remained constant when a sequestered trace mineral source was used. A decrease of 10% activity was found when a chealated trace mineral source was used.

The suspensibility of iron magnesium, and zinc were studied for the two trace mineral sources. It was found that for iron and zinc the sequestered minerals remained constant thoughout storage. The magnesium concentration increased at the bottom of the storage tanks for both mineral sources studied.

APPENDIX A

TABLE I

pH During Delvocid Storage Study

Sample	WK 0	WK 2	WK 4	WK 8
JD 1 A	3.71	3.82	3.82	3.82
B	3.73	3.83	3.82	3.82
JD 2 A	3.72	3.83	3.84	3.83
B	3.73	3.84	3.83	3.82
JD 3 A	3.73	3.81	3.82	3.82
B	3.74	3.80	3.82	3.82
JD 4 A	3.71	3.82	3.80	3.81
B	3.73	3.81	3.81	3.81
JD 5 A	3.70	3.80	3.80	3.81
B	3.70	3.81	3.81	3.81
JD 6 A	3.70	4.81	5.30	5.40
B	3.71	4.82	5.31	5.40

TABLE II

Crude Protein During Delvocid Storage Study

Sample	WK 0 As Is	WK 0 Dry Basis	WK 2 As Is	WK 2 Dry Basis	WK 4 As Is	WK 4 Dry Basis	WK 8 As Is	WK 8 Dry Basis
JD 1 A	66.05	155.30	65.20	152.19	65.92	157.70	67.43	160.01
B	64.94	155.14	65.73	155.76	65.53	157.71	66.28	157.92
JD 2 A	65.22	155.40	64.73	155.00	64.94	156.71	66.66	161.56
B	66.14	158.34	66.03	157.10	65.66	158.79	66.10	160.43
JD 3 A	65.35	156.19	63.90	155.44	65.04	157.35	66.26	158.06
B	66.39	158.15	65.52	156.52	65.93	157.80	67.16	158.58
JD 4 A	64.81	156.47	63.98	155.06	64.85	154.48	65.72	157.30
B	66.44	157.77	65.32	155.63	64.70	156.13	66.85	161.16
JD 5 A	63.61	152.29	64.36	153.60	65.14	154.07	66.05	157.98
B	65.68	156.27	67.39	158.34	65.26	157.82	66.61	159.39
JD 6 A	64.15	153.03	64.04	152.59	64.07	152.77	64.77	156.87
B	65.26	155.16	66.13	156.59	66.37	156.23	65.51	155.24

TABLE III

Dry Matter During Delvocid Storage Study

Sample	WK 0	WK 2	WK 4	WK 8
JD 1 A	42.53	42.84	41.80	42.14
B	41.86	42.20	41.55	41.97
JD 2 A	41.97	41.76	41.44	41.26
B	41.77	42.03	41.35	41.20
JD 3 A	41.84	41.11	41.34	41.92
B	41.98	41.86	41.78	42.35
JD 4 A	41.42	41.26	41.98	41.78
B	42.11	41.97	41.44	41.48
JD 5 A	41.77	41.90	42.28	41.61
B	42.03	42.56	41.35	41.79
JD 6 A	41.92	41.97	41.94	41.29
B	42.06	42.23	42.48	42.20

TABLE IV

Maltose Equivalents During Delvocid Storage Study

Sample	WK 0		WK 2		WK 4		WK 8	
	As Is	Dry Basis	As Is	Dry Basis	As Is	Dry Basis	As Is	Dry Basis
JD 1 A	95.20	223.84	109.17	254.83	96.20	230.14	96.75	229.59
B	98.17	234.52	100.35	237.80	93.74	225.61	97.80	233.02
JD 2 A	95.53	227.61	97.13	232.59	96.96	233.98	97.90	237.28
B	100.22	239.93	97.84	232.79	94.41	228.32	93.02	225.78
JD 3 A	96.42	230.45	98.09	238.60	99.59	240.91	96.34	229.82
B	100.48	239.35	98.92	236.31	97.94	234.42	96.92	228.85
JD 4 A	92.62	223.61	95.52	231.51	96.49	229.85	92.21	220.70
B	100.22	237.99	97.01	231.14	95.57	230.82	96.70	233.31
JD 5 A	93.61	224.11	98.62	235.36	100.46	237.60	96.92	231.01
B	100.58	239.30	99.95	234.84	90.01	234.61	97.85	234.15
JD 6 A	100.91	240.73	100.61	239.72	100.09	240.58	100.39	243.13
B	97.38	241.04	97.55	231.00	96.91	228.13	97.35	230.68

TABLE V

Free Ammonia During Delvocid Storage Study

Sample	WK 0		WK 2		WK 4		WK 8	
	As Is	Dry Basis	As Is	Dry Basis	As Is	Dry Basis	As Is	Dry Basis
JD 1 A	1.488	3.499	1.590	3.712	1.603	3.835	1.629	3.865
B	1.419	3.390	1.495	3.543	1.523	3.666	1.442	3.735
JD 2 A	1.826	4.351	1.755	4.203	1.592	3.842	1.610	3.902
B	1.645	3.938	1.548	3.683	1.532	3.705	1.539	3.735
JD 3 A	1.868	4.465	1.971	4.795	1.853	4.482	1.883	4.492
B	1.854	4.416	1.973	4.713	1.645	3.937	1.685	3.977
JD 4 A	1.665	4.020	1.763	4.273	1.745	4.157	1.757	4.206
B	1.660	3.942	1.975	4.706	1.484	3.581	1.494	3.602
JD 5 A	1.581	3.785	1.591	3.797	1.603	3.791	1.597	3.820
B	1.571	3.738	1.754	4.121	1.832	4.430	1.861	4.529
JD 6 A	2.345	5.594	2.413	5.749	5.928	14.134	6.253	15.144
B	2.355	5.599	2.884	6.829	5.876	13.832	6.304	14.930

TABLE VI

Viscosity During Delvocid Storage Study

Sample	WK 0	WK 2	WK 4	WK 8
JD 1 A	788	813	780	800
B	759	798	785	791
JD 2 A	775	815	723	800
B	733	800	735	755
JD 3 A	780	800	788	790
B	743	800	743	790
JD 4 A	782	802	738	790
B	717	738	732	723
JD 5 A	763	815	813	800
B	733	802	810	794
JD 6 A	773	825	798	800
B	740	788	790	795

TABLE VII

Effect of Delvocid on Protein Synthesis of Stored Samples on a Dry Matter Protein Basis

(Reported as Percent of Control[A])

Sample	Run 1 Week 0	Run 2 Week 0	Run 1 Week 2	Run 2 Week 2	Run 1 Week 4	Run 2 Week 4	Run 1 Week 8	Run 2 Week 8
JD 1 A	82	92	76	114	96	121	91	85
B	104	90	133	123	98	73	80	81
JD 2 A	88	99	82	80	106	127	84	102
B	92	88	104	112	97	98	86	96
JD 3 A	75	113	99	118	105	125	88	104
B	97	102	94	123	71	72	93	89
JD 4 A	81	108	92	116	106	104	77	77
B	96	107	125	128	110	102	96	85
JD 5 A	100	100	100	100	100	100	100	100
B	100	100	100	100	100	100	100	100
JD 6 A	90	108	97	130	104	117	99	91
B	99	94	109	91	74	86	109	93

TABLE VIII

Effect of Storage on IN VITRO Protein Synthesis

on a Percent Dry Matter Protein Basis

(Reported as Percent of Control[A])

Sample	Run 1 Week 0	Run 2 Week 0	Run 1 Week 2	Run 2 Week 2	Run 1 Week 4	Run 2 Week 4	Run 1 Week 8	Run 2 Week 8
JD 1 A	92	92	78	88	91	100	92	73
B	107	96	122	136	132	84	93	87
JD 2 A	99	91	83	62	101	105	85	80
B	95	95	95	123	131	114	112	103
JD 3 A	85	104	102	91	100	104	89	87
B	99	110	86	135	96	84	115	96
JD 4 A	91	100	95	89	101	91	78	89
B	99	113	112	141	141	118	85	91
JD 5 A	113	91	103	76	95	83	101	94
B	103	105	95	110	135	116	110	108
JD 6 A	100	100	100	100	100	100	100	100
B	100	100	100	100	100	100	100	100

[A] Control is sample JD 6 A&B which is pressure cooked sample with no preservative added.

APPENDIX B

TABLE I

Effect of Storage on the Chemical Activity of Na-Monensin[A]

TRT	REP	Level[B]	Week[B] 0	3	4	6	8
1	1	1	398.0	395.8	403.5	403.5	400.0
		2	400.0	406.2	398.2	395.8	398.2
		3	401.0	414.0	406.7	401.0	401.0
		4	397.0	395.8	398.2	398.2	400.0
		5	398.0	403.0	403.6	402.0	398.2
1	2	1	400.0	395.8	400.3	400.3	399.2
		2	398.0	412.6	398.2	397.4	398.2
		3	397.0	399.5	406.7	405.5	400.0
		4	401.0	399.5	398.2	399.6	397.5
		5	402.0	390.6	403.5	394.2	402.0
2	1	1	400.0	406.2	403.5	397.8	401.5
		2	398.0	395.8	418.0	398.5	399.0
		3	397.0	414.0	398.2	399.0	398.5
		4	400.0	403.0	403.5	400.0	400.0
		5	402.0	402.0	406.7	401.5	399.6
2	2	1	400.0	411.5	398.2	386.3	398.5
		2	401.0	395.8	416.0	398.5	398.5
		3	398.0	403.0	418.0	402.3	400.2
		4	402.0	405.3	406.7	401.2	401.0
		5	400.0	403.0	406.7	399.6	399.0
3	1	1	400.0	411.5	399.5	399.5	401.3
		2	397.0	403.0	393.4	393.4	400.0
		3	401.0	395.8	396.3	398.2	398.2
		4	400.0	411.0	386.4	387.6	399.3
		5	402.0	395.8	399.5	399.5	397.6
3	2	1	397.0	403.0	408.6	402.3	401.4
		2	399.0	414.0	399.5	398.6	399.8
		3	406.0	395.8	396.5	397.4	397.6
		4	398.0	411.5	390.1	392.6	401.2
		5	402.0	403.0	411.6	402.3	399.6
4	1	1	398.0	403.0	390.0	387.2	402.7
		2	400.0	402.5	390.0	389.2	400.0
		3	401.0	411.0	381.4	390.0	398.6
		4	398.0	404.8	398.5	399.2	397.8
		5	400.0	405.3	390.0	394.3	399.0
4	2	1	400.0	403.0	403.6	401.2	397.6
		2	398.0	412.5	402.5	400.0	401.2
		3	400.0	399.0	398.5	397.6	398.7
		4	401.0	411.7	396.3	398.4	400.0
		5	400.0	395.8	399.5	400.0	392.0

[A] Na-Monensin activity is expressed in mg/lb of supplement.

[B] Weekly level Activity is reported as the average of duplicate determinations.

TABLE II

Effect of Storage on Vitamin A[A] Activity and Suspension

TRT	REP	Level	Week[B] 0	2	4	6	8
1	1	1	130	130	125	118.5	117.0
		2	130	132.8	127.5	117.0	119.5
		3	130.5	131.5	119.5	115.5	115.0
		4	129.5	130.0	125.0	120.8	120.8
		5	130.0	129.0	127.6	110.0	115.0
1	2	1	130.0	130.0	127.5	119.6	111.7
		2	130.0	127.5	127.0	120.8	113.3
		3	130.5	134.0	120.7	118.0	118.0
		4	130.0	132.8	127.0	107.2	111.7
		5	130.5	132.8	119.5	119.0	111.7
2	1	1	130.0	130.0	125.0	120.8	117.0
		2	130	134.5	119.5	117.0	119.5
		3	130.5	132.8	125.5	115.5	117.0
		4	130.0	127.5	125.0	118.0	120.8
		5	130.0	132.8	118.5	119.0	113.3
2	2	1	130.0	130.0	118.5	115.5	117.0
		2	129.5	129.5	108.0	112.8	117.0
		3	130.5	130.0	125.0	120.0	118.0
		4	130.0	133.0	114.0	119.5	119.5
		5	130.5	132.8	120.8	118.0	118.0
3	1	1	130.0	132.8	122.0	120.0	119.0
		2	130.0	134.0	122.6	121.0	118.0
		3	130.5	127.5	130.4	112.8	119.0
		4	130.0	130.0	126.6	115.8	117.0
		5	130.0	130.0	126.6	119.3	118.0
3	2	1	130.0	134.0	133.5	120.8	120.0
		2	130.0	129.5	130.5	126.5	123.0
		3	130.5	130.0	132.5	115.9	119.5
		4	130.5	127.5	126.0	122.0	118.0
		5	130.5	130.0	137.3	120.8	119.5
4	1	1	130.5	132.5	125.5	123.7	120.8
		2	129.5	130.0	124.0	126.5	123.0
		3	130.0	133.0	122.0	123.7	122.0
		4	130.0	134.0	122.0	123.0	123.0
		5	130.0	130.0	126.0	126.0	123.3
4	2	1	130.5	131.5	108.0	128.0	120.8
		2	129.5	132.0	127.3	129.5	122.3
		3	130.0	131.5	124.4	125.0	123.4
		4	130.0	130.0	126.6	127.3	122.0
		5	130.0	129.5	126.6	125.0	125.0

[A] Vitamin A acitvity is reported as ug/g.

[B] The weekly activity of Vitamin A is reported as the average of duplicate determinations.

APPENDIX C

Table I

Iron[A] Concentration During Storage

TRT	REP	Level	Week of Storage 0	2	4	6	8
1	1	Top	281	282	310	260	362
		Middle	307	242	314	354	374
		Bottom	327	258	280	254	304
1	2	Top	287	338	310	279	330
		Middle	328	329	257	282	261
		Bottom	311	307	332	392	236
2	1	Top	300	327	309	281	282
		Middle	360	300	379	266	328
		Bottom	307	279	360	236	363
2	2	Top	357	260	380	318	356
		Middle	359	270	323	307	390
		Bottom	370	386	307	190	364
3	1	Top	291	262	339	311	366
		Middle	332	318	327	369	329
		Bottom	293	278	258	264	344
3	2	Top	292	339	254	295	330
		Middle	300	285	214	321	289
		Bottom	305	255	231	249	313
4	1	Top	228	204	199	214	271
		Middle	256	258	186	220	218
		Bottom	250	230	256	256	246
4	2	Top	251	445	225	252	269
		Middle	259	258	264	258	254
		Bottom	290	241	218	247	266

[A] Iron concentration expressed in ppm on a dry matter basis.

Table II

Magnesium[A] Concentration During Storage

TRT	REP	Level	Week of Storage 0	2	4	6	8
1	1	Top	670	753	701	634	515
		Middle	680	599	722	485	663
		Bottom	708	723	721	753	795
1	2	Top	683	590	716	677	642
		Middle	722	645	577	602	657
		Bottom	729	755	695	740	527
2	1	Top	717	580	686	659	647
		Middle	677	673	531	683	589
		Bottom	658	737	677	701	365
2	2	Top	645	717	661	716	697
		Middle	642	705	630	675	392
		Bottom	699	703	582	485	411
3	1	Top	684	504	755	502	312
		Middle	668	613	612	459	423
		Bottom	596	705	630	620	433
3	2	Top	690	559	628	577	503
		Middle	648	600	602	648	482
		Bottom	650	706	657	529	457
4	1	Top	653	634	586	567	515
		Middle	586	645	633	683	435
		Bottom	600	588	575	411	414
4	2	Top	640	635	650	474	353
		Middle	569	579	645	564	407
		Bottom	567	564	628	613	431

[A] Magnesium concentration is expressed in ppm on a dry matter basis.

Table III

Zinc[A] Concentration During Storage

TRT	REP	Level	Week of Storage 0	2	4	6	8
1	1	Top	264	269	261	146	278
		Middle	268	220	279	284	303
		Bottom	277	262	248	159	207
1	2	Top	259	220	278	188	188
		Middle	281	186	134	246	235
		Bottom	268	259	340	326	216
2	1	Top	284	187	227	201	217
		Middle	277	199	339	172	214
		Bottom	182	221	392	219	288
2	2	Top	206	230	336	201	294
		Middle	215	242	192	296	299
		Bottom	268	249	191	139	295
3	1	Top	264	157	249	270	259
		Middle	254	190	327	314	288
		Bottom	182	232	167	192	288
3	2	Top	181	203	171	356	307
		Middle	216	202	166	383	280
		Bottom	219	246	157	263	277
4	1	Top	243	241	275	182	232
		Middle	249	203	253	268	225
		Bottom	276	277	234	231	228
4	2	Top	216	223	267	237	232
		Middle	213	207	257	239	235
		Bottom	216	272	233	253	230

[A] Zinc concentration is expressed in ppm on a dry matter basis.

APPENDIX D

TABLE I

STATISTICAL ANALYSIS OF pH DURING STORAGE

Source of Variation	Degrees of Freedom	Mean Square	F Value	Probability of a greater F
Corrected Total	47			
TRT	5	1.3701	25372.22	.0001
Error A	6	.000054		
WEEK	3	.3399	8497.5	.0001
TRT*WEEK	15	.1787	4467.5	.0001
Error B	18	.00004		

TABLE 2

STATISTICAL ANALYSIS OF pH, WITH TREATMENT JD 6 REMOVED, DURING STORAGE

Source of Variation	Degrees of Freedom	Mean Square	F Value	Probability of a greater F
Corrected Total	39			
TRT	4	.000715	16.82	.0001
Error A	5	.0000425		
WEEK	3	.023522	480.04	.0001
TRT*WEEK	12	.0001183	2.41	.0553
Error B	15	.000049		

TABLE 3

STATISTICAL ANALYSIS OF CRUDE PROTEIN (AS IS BASES). DURING STORAGE

Source of Variation	Degrees of Freedom	Mean Square	F Value	Probability of a greater F
Total	47			
TRT	5	.7329	.298	0.8972
Error A	6	2.4565		
WEEK	3	3.1120	8.507	0.001
TRT*WEEK	15	.4003	1.094	.4228
Error B	18	.3658		

TABLE 4

STATISTICAL ANALYSIS OF CRUDE PROTEIN (DRY MATTER BASES) DURING STORAGE

Source of Variation	Degrees of Freedom	Mean Square	F Value	Probability of a Larger F Value
Total	47			
TRT	5	9.4493	1.545	0.3040
Error A	6	6.1179		
WEEK	3	29.5496	18.277	0.0001
TRT*WEEK	15	1.7212	1.165	0.4444
Error B	18	1.6168		

TABLE 5

STATISTICAL ANALYSIS OF DRY MATTER DURING STORAGE

Source of Variation	Degrees of Freedom	Mean Square	F Value	Probability of a Greater F Value
Total	47			
TRT	5	.3205	1.56	.2990
Error A	6	.2049		
WEEK	3	.1812	1.86	.1932
TRT*WEEK	15	.1289	1.32	.2839
Error B	18	.0976		

TABLE 6

STATISTICAL ANALYSIS OF STARCH DAMAGE (AS IS BASES) DURING STORAGE

Source of Variation	Degrees of Freedom	Mean Square	F Value	Probability of a Greater F Value
Total	47			
TRT	5	10.996	1.32	.3684
Error A	6	8.337		
WEEK	3	18.431	1.90	.1663
TRT*WEEK	15	6.261	.64	. 8026
Error B	18	9.715		

TABLE 7

STATISTICAL ANALYSIS OF STARCH DAMAGE
(DAY BASES) DURING STORAGE

Source of Variation	Degrees of Freedom	Mean Square	F Value	Probability of a Large F Value
Total	47			
TRT	5	45.227	1.02	.4818
Error A	6	44.460		
WEEK	3	50.239	1.35	.2912
TRT*WEEK	15	32.163	.86	.6110
Error B	18	37.349		

TABLE 8

STATISTICAL ANALYSIS OF FREE AMMONIA
(AS IS BASES) DURING STORAGE

Source of Variation	Degrees of Freedom	Mean Square	F Value	Probability of a Greater F Value
Total	47			
TRT	5	9.254	319.10	.0001
Error A	6	.029		
WEEKS	3	1.263	105.25	.0001
TRT*WEEKS	13	1.497	124.75	.001
Error B	18	.0120		

TABLE 8 - Continued

TREATMENT 6 REMOVED (NEGATIVE CONTROL)

Source of Variation	Degrees of Freedom	Mean Square	F Value	Probability of a Greater F Value
Total	39			
TRT	4	.106	3.66	.0921
Error A	5	.029		
WEEKS	3	.0217	2.41	.1023
TRT*WEEKS	12	.013	1.49	.2176
Error B	15	.009		

TABLE 9

STATISTICAL ANALYSIS OF FREE AMMONIA (DRY MATTER BASES) DURING STORAGE

Source of Variation	Degrees of Freedom	Mean Square	F Value	Probability of a Greater F Value
Total	47			
TRT	5	52.023	358.78	.0001
Error A	6	.145		
WEEKS	3	7.574	103.10	.0001
TRT*WEEK	13	8.476	116.11	.001
Error B	18	.073		

TABLE 10

STATISTICAL ANALYSIS OF VISCOSITY DURING STORAGE

Source of Variation	Degrees of Freedom	Mean Square	F Value	Probability of a Greater F Value
Total	47			
TRT	5	1,651.28	1.02	.4793
Error A	6	1,614.00		
WEEK	3	3,270.97	10.18	.0004
TRT*WEEK	15	600.97	1.87	.1031
Error B	18	321.33		

TABLE 11

STATISTICAL ANALYSIS OF PROTEIN SYNTHESIS
DURING STORAGE TREATMENT 5 AS CONTROL

Source of Variation	Degrees of Freedom	Mean Square	F Value	Probability of a Greater F Value
Total	46			
TRT	4	592.8	4.88	.001
RUN	1	1407.45	11.59	.001
REP (TRT)	5	1344.25	11.07	.001
Error	36	121.47		
	Treatment 6 as Control			
Total	46			
TRT	4	603.28	4.73	.001
RUN	1	1507.45	11.83	.001
REP (TRT)	5	1388.25	10.89	.001
Error	36	127.47		

TABLE 12

STATISTICAL ANALYSIS OF MONENSIN CHEMICAL ACTIVITY DURING STORAGE

Source of Variation	Degrees of Freedom	Mean Square	F Value	Probability of a Greater F Value
TRT	3	80.43	1.65	.3137
Error A	4	48.86		
WEEK	4	187.84	7.40	.0014
WEEK*TRT	12	67.26	2.65	.0355
Error B	16	25.38		
Level	4	2.39	.16	.9597
TRT*Level	12	30.00	1.96	.0389
WEEK*Level	16	23.34	1.53	.1109
TRT*WEEK*Level	48	32.53	2.13	.0014
Error C	80	15.29		

TABLE 13

STATISTICAL ANALYSIS OF VITAMIN A ACTIVITY DURING STORAGE

Source of Variation	Degrees of Freedom	Mean Square	F Value	Probability of a Greater F Value
TRT	3	87.71	4.25	.0981
Error A	4	20.66		
Week	4	1129.60	31.14	.0001
Week*TRT	12	89.93	2.48	.0460
Error B	16	36.27		
Level	4	19.83	.67	.6175
Level*TRT	12	28.56	.96	.4940
Level*Week	16	18.70	.63	.8519
Level*Week*TRT	48	23.91	.80	.7925
Error C	80	29.77		

Table XIV

Analysis of Variance for Iron, Magnesium, and Zinc Concentration During Storage

Dependent Variable Iron:

Source of Variation	Degrees of Freedom	Mean Square	F Value	Probability of a Greater F Value
TRT	3	29694.7	7.83	0.0377
Error A	4	3792.2		
Week	4	4964.25	2.85	0.0585
Week*TRT	12	2936.6	1.71	0.1555
Error B	16	1742.04		
Level	2	1729.5	1.05	.3582
Level*TRT	6	687.6	0.42	.8621
Level*Week	8	1714.1	1.04	.4205
Level*Week*TRT	24	1256.35	0.77	.7542
Error C	40	1642.0		

Dependent Variable Magnesium:

Source of Variation	Degrees of Freedom	Mean Square	F Value	Probability of a Greater F Value
TRT	3	171386.5	28.09	.0038
Error A	4	2541.8		
Week	4	98911.2	36.17	.0001
Week*TRT	12	7243.5	2.65	.0356
Error B	16	2734.9		
Level	2	3082.2	.6	.5540
Level*TRT	6	11135.8	2.17	.0667
Level*Week	8	4324.9	.84	.5725
Level*Week*TRT	24	5957.0	1.16	.3326
Error C	40	5066.4		

Dependent Variable Zinc:

Source of Variation	Degrees of Freedom	Mean Square	F Value	Probability of a Greater F Value
TRT	3	229.3	1.43	.3586
Error A	4	160.5		
Week	4	3056.3	.96	.4551
Week*TRT	12	5649.1	1.78	.1403
Error B	16	3178.6		
Level	2	748.3	.35	.7083
Level*TRT	6	1597.7	.71	.6446
Level*Week	8	3036.5	1.35	.2495
Level*Week*TRT	24	1393.5	.62	.8936
Error C	40	2255.0		

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DELVOCID® AND SODIUM CHLORIDE EFFECTS ON THE STABILITY OF A CORN BASED LIQUID SUPPLEMENT CONTAING UREA, MONENSIN, VITAMIN A AND TRACE MINERALS

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AN ABSTRACT OF A MASTER'S THESIS

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ABSTRACT OF THESIS

Two storage trials were conducted to determine: (1) if the product Delvocid could be used as a preservative in a corn based liquid supplement; (2) to determine what steps need to be taken to maintain Na-Monensin's chemical activity in a corn based liquid stored at a pH of 3.6; (3) to determine the stability of Vitamin A palmitate in this liquid supplement when two different types of trace minerals were used; (4) to determine the suspensibility of trace mineral from the two sources used.

In study one levels 150, 200, and 250 ppm of Delvocid were compared to a positive control (5,000 ppm proprionic acid) and to negative control (no preservative added). The parameters pH, crude protein, dry matter, ammonia release, maltose equivalence, protein synthesis, and viscosity were monitored during the study. Results indicated that either the 200 or the 250 ppm level of Delvocid was able to maintain constant values during the trial and it compared well with the proprionic acid control.

Preliminary investigation on the effect of NaCl addition on Na-Monensin chemical activity indicated that a level of 5 or 10% was capable of maintaining activity for 24 hours. To investigate the ability of 5% NaCl addition on the chemical activity during storage and to determine the stability of Vitamin A palmitate and the effects of trace mineral supplements on these feed additives a second storage trial was run. The results of this study indicated that Na-Monensin's chemical activity was maintained during 8 weeks